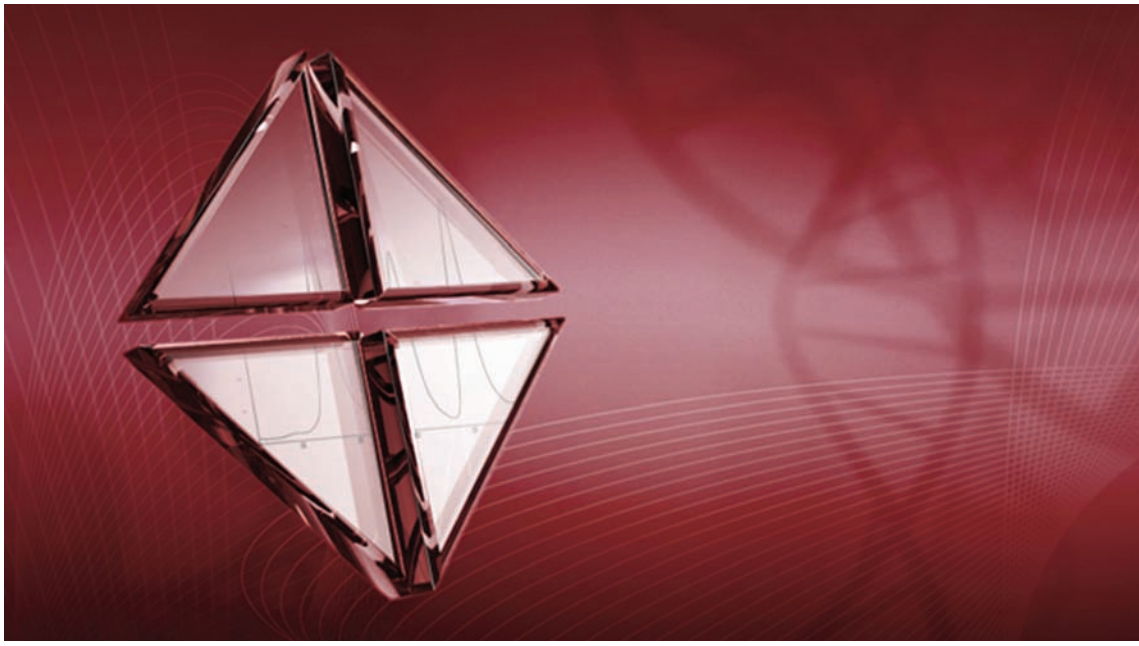
GE Healthcare

UNICORN™ 6

Evaluation Manual



UNICORN

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1 Introducing UNICORN Evaluations

Introduction

This chapter contains:

- A general introduction to the evaluation of results using UNICORN 6 software.
- Information about the user documentation for UNICORN 6, including an overview of related documents describing the use of the software.

Software declaration of conformity

UNICORN 6 is technically compatible with all relevant sections of FDA 21 CFR Part 11.

A part 11-system assessment checklist is available on request through the local GEHC representative.

In this chapter

This chapter contains these sections:

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1.1 About evaluation of UNICORN results

Introduction

This section is a brief introduction to the evaluation of results created in UNICORN and a description of the scope of this manual.

What is UNICORN?

UNICORN is a complete software package for:

- control and supervision of chromatography systems.
- evaluation and analysis of the results from separation runs.

This manual describes UNICORN 6, which is designed for ÄKTAavant™ chromatography systems. This software version is not compatible with other ÄKTA™ systems.

Workflow

The workflow in UNICORN can be divided into four distinct stages. The flow chart below shows the work flow stages.

This manual describes steps 3 and 4 of this workflow.

TIP: Step 1, how to create and optimize methods, is described in the *"UNICORN 6 Method Manual"*. Step 2, how to perform method runs, is described in the *"ÄKTAavant and UNICORN 6 User Manual"*.

Evaluate the results

A result file is automatically generated at the end of a method run and contains a complete record of the method run, including method, system settings, curve data and method run log. The data monitored during the performing of the run are recorded in the UNICORN database. A result file from a complete run contains a chromatogram (or several chromatograms) with monitor data represented graphically by individual curves.

Using the UNICORN ***Evaluation*** module you can view and analyze result data and optimize the presentation of the results, for example by:

- integrating peaks
- measuring peak data
- comparing curves and chromatograms from different results

  etc.

Note: *The source result data created during the run are always saved regardless of your editing operations. Edited curves are saved as copies, with the original source curve uncompromized. The original result curves cannot be deleted or overwritten.*

Compile a report

When you are satisfied with the result, you may compile a report. The report can be anything from a simple print of a chromatogram, showing selected curves, to a comprehensive report containing, for example:

- chromatograms from several results
- run data in text form
- log entries
- images

The report options are described in *Section 3.6 Generate reports, on page 70*.

1.2 About this manual

Introduction

This section describes the purpose of the manual, the general structure and conventions applied in the text, and some prerequisites that should be fulfilled before you start to apply any of the procedures described in the following chapters.

The purpose of the UNICORN Evaluation Manual

The purpose of the UNICORN Evaluation Manual is to provide a comprehensive guide to the evaluation features of the UNICORN software. The ***Evaluation*** module of the software is presented, along with practical instructions on how to use the functions.

Basically the manual covers the following, major topics:

- how to view chromatographic results and how to compile result reports
- how to edit and optimize the representation of the results

  and
- how to automate frequent evaluation procedures.

The manual also describes algorithms and theoretical models used in the evaluations.

Note: *The Evaluation Manual does not describe the functions of every command in all panels and dialogs of the user interface. Refer to the online user interface help for information about commands that are not described in this manual. The online help in the Evaluation module is accessed either by clicking Help buttons in software dialogs or by pressing the* ***F1*** *key.*

Document structure

Each chapter starts with a brief overview that presents the contents and the headings for the sections that the chapter contains. Most sections begin with an introduction that summarizes the content. Some sections are divided into sub-sections, each with an overview of the contents.

A section is divided into blocks of information with separating lines. The blocks are identified by a label extending into the margin (such as the label Document Structure above). This makes it easier for you to quickly scan a page to find the exact topic you are looking for.

Typographical conventions

Menu commands, field names and other text items from the software are quoted exactly as they appear on the screen, in a bold italic typeface:

Example: ***Result Navigator***

Search paths are shown in a bold italic typeface with a separating colon between each level:

Example: ***Edit:Delete:Curves*** i.e., the menu option ***Curves*** in the sub-menu ***Delete*** from the ***Edit***-menu.

Controls on the instrument, computer or keyboard keys are shown with a bold, regular typeface:

Example: Press the **Delete** key.

Text that the user must either type exactly as shown in the manual, or that UNICORN displays as a response (not a regular part of the graphic user interface), is represented by a monotype typeface within quotation marks:

Example: "Connection change"

Prerequisites

The following prerequisites must be fulfilled before you can use this manual the way it is intended:

- You need to have a general understanding of how your PC and Windows work. In most cases universal computer functions will not be explained.
- UNICORN must be installed and configured correctly on your computer.
- Your user profile and access rights must be set up, and you must be able to log on to UNICORN, access a database and result files.
- You need to understand the general concepts of liquid chromatography. Terminology and functionalities will be explained only when they differ from normal practice.

1.3 UNICORN 6 user documentation

Introduction

This section describes the user documentation that is delivered with an ÄKTAavant system.

User documentation

The user documentation listed in the table below is available from the ***Help*** menu in UNICORN or on the ÄKTAavant and UNICORN User Documentation CD.

Document	Main contents
ÄKTAavant and UNICORN 6 Installation Guide	Site preparation, stand-alone installation and test procedure.
Getting Started with ÄKTAavant and UNICORN 6	System overview and instructions to perform a basic run.
ÄKTAavant and UNICORN 6 User Manual	Instructions for safe handling of the system. Descriptions of components. Information about how to run and maintain the system.
UNICORN Help	Dialog descriptions for UNICORN (from the ***Help*** menu).
UNICORN 6 Method Manual	Overview and detailed descriptions of the method creation features in UNICORN. Instructions on how to use the software. Workflow descriptions for common operations.
UNICORN 6 Evaluation Manual	Overview and detailed descriptions of the evaluation features in UNICORN. Instructions on how to use the software. Workflow descriptions for common operations.
UNICORN 6 Administration and Technical Manual	Network setup and complete software installation. Administration of UNICORN and the UNICORN database.

2 The UNICORN Evaluation module

Introduction

This chapter is an overview of the UNICORN ***Evaluation*** module with descriptions of some of the elements of the user interface.

Note: *A user may be assigned to an* ***Access Group*** *which is set up so that the user only has limited access to the various UNICORN modules. The modules that are unavailable cannot be selected or opened when the program is started.*

In this chapter

This chapter contains this section:

Section	See page
2.1 Evaluation module	12

2.1 Evaluation module

The Evaluation module interface

The interface consists of a pane where the current result is displayed and the ***Result Navigator***. Both panes are described below.

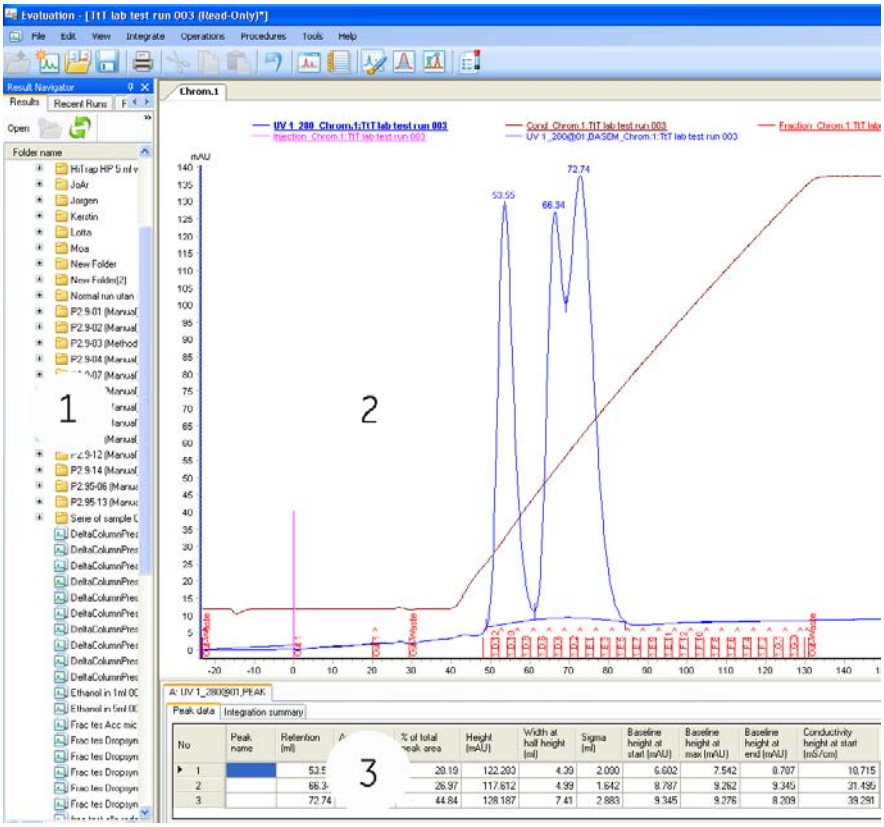
Evaluation module window

The illustration below shows the different parts of the ***Evaluation*** module with a result displayed:

1 ***Result Navigator***

2 ***Chromatogram*** pane

3 ***Peak table***



Toolbar icons in the Evaluation module

The table below describes the toolbar icons in the ***Evaluation*** module.

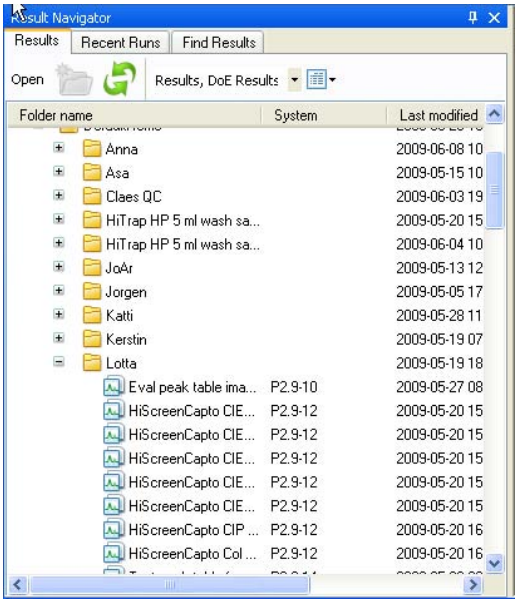
Icon	Function
	Opens the ***Result Navigator***. The ***Result Navigator*** is described below.
	Opens a new, empty result file. ***Note:*** *This function can also be selected in the **File** menu.*
	Opens the ***Open Curves To Compare*** dialog. You may use the dialog to search for curves to compare in specified folders, results or chromatograms. This dialog is described in *Section 4.5 Compare different runs, on page 159*. ***Note:*** *This dialog can also be opened from the **File** menu.*
	Saves the current result. If the current result is new and has not been saved yet, the ***Save As*** dialog will open so that the result can be named and saved in a selected folder. ***Note:*** *This function can also be selected in the **File** menu.*
	Opens the ***Print Chromatograms*** dialog. This dialog is used for printing the chromatograms on a selected printer. There are options for the print layout and Preview before printing. This dialog is described in *Section 3.4 Print chromatograms and peak data, on page 57*. ***Note:*** *This dialog can also be opened from the **File** menu.*
	Cuts the selected result or folder from the current place in the file structure. ***Note:*** *This function is available only for items in the **Result Navigator** pane. Cut can also be selected in the **Edit** menu.*

Icon	Function
	• Copies the selected result or folder, when the ***Result Navigator*** is selected. or • Opens the ***Copy to Clipboard*** dialog, when an open result is selected. Either the curve window or the peak table data may be copied to the Windows clipboard. ***Note:*** *This function can also be selected in the **Edit** menu.*
	Pastes a copied result or folder to the selected folder. ***Note:*** *This function is available only for items in the **Result Navigator** pane. Paste can also be selected in the **Edit** menu.*
	Returns the result to the state it was in before the last change (***Undo***). ***Note:*** *This function can also be selected in the **Edit** menu.*
	Opens the ***Generate Report*** dialog. The ***Generate Report*** dialog is used to preview and print reports in selected, saved formats and also to delete saved formats. When ***New*** is selected, or a saved format is selected for editing in the ***Generate Report*** dialog, the ***Customize Report*** window is opened in the ***Evaluation*** module. The editing process is described in detail in *Section 3.6 Generate reports, on page 70*. ***Note:*** *This dialog can also be opened from the **File** menu.*
	Opens the ***Documentation*** dialog. The dialog contains the complete documentation for the result, including for example the ***Run Log***, ***Evaluation Log*** and ***System Settings***. This dialog is described in *Section 3.5 Run documentation, on page 60*. ***Note:*** *This dialog can also be opened from the **View** menu.*
	Opens the ***Customize*** dialog where curve and axis settings, styles and colors, header content and text items can be set. Saved layouts can be selected and edited layouts can be saved for later use. This dialog and the layout operations are described in *Section 3.3 Optimize the presentation of chromatograms, on page 36*. ***Note:*** *This dialog can also be opened by selecting **Customize** from the chromatogram right-click shortcut menu.*

Icon	Function
	Opens the ***Peak Integrate*** dialog, where curve characteristics including peak areas, retention time and peak widths are identified and measured. This dialog is described in detail in *Section 4.2 Peak integration, on page 98*. ***Note:*** *This dialog can also be opened from the* ***Integrate*** *menu.*
	Opens the ***Multi Result Peak Compare*** wizard. This wizard can be used to compare peak data from different results, for example from a complete series of ***Scouting*** runs. The options and how to use the wizard is described in *Section 4.6 The Multi Result Peak Compare wizard, on page 179*. ***Note:*** *This dialog can also be opened from the* ***File*** *menu.*
	Opens the ***Column Handling*** dialog, where information about the columns is viewed and edited.

The Result Navigator pane

The ***Result Navigator*** shows all the user folders, individual results and ***Design of Experiment*** results that are available. Using the tool icons, ***Edit*** menu commands or the right-click menu, results may for example be copied, deleted, or renamed.



Note: *The **Result Navigator** pane features are described in Section 3.2 Locate results, on page 27.*

Result Navigator Auto Hide

The ***Result Navigator*** may either be displayed open in the left portion of the ***Evaluation*** module, or the ***Auto Hide*** function can be selected by clicking the pin symbol in the top right-hand corner of the pane.

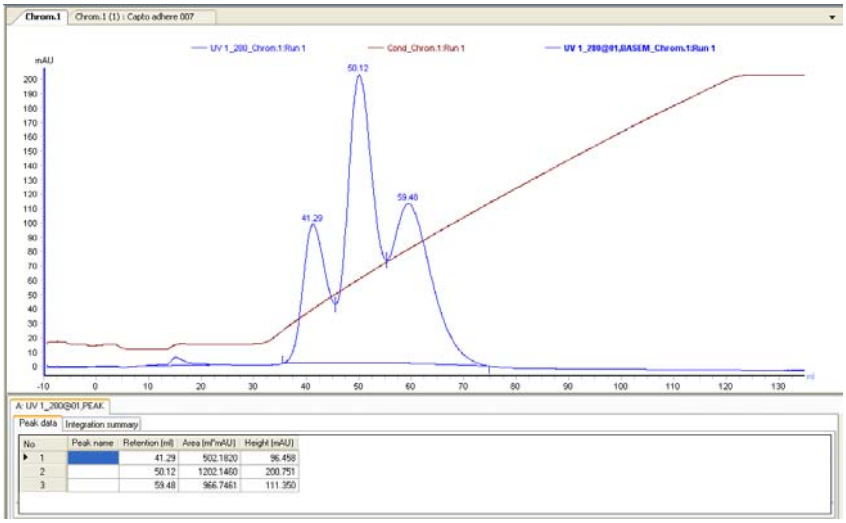
Pin direction	Function
	Auto Hide is off. Click the pin symbol to turn the function on.

Pin direction	Function
	Auto Hide is on. Click the pin symbol to turn the function off.

If ***Auto Hide*** is selected, the ***Result Navigator*** opens automatically when the mouse pointer is placed over its tab. It remains open as long as the mouse pointer remains over the pane. The pane closes automatically when the pointer is moved outside.

The Chromatogram pane

The illustration below shows the ***Chromatogram*** pane.



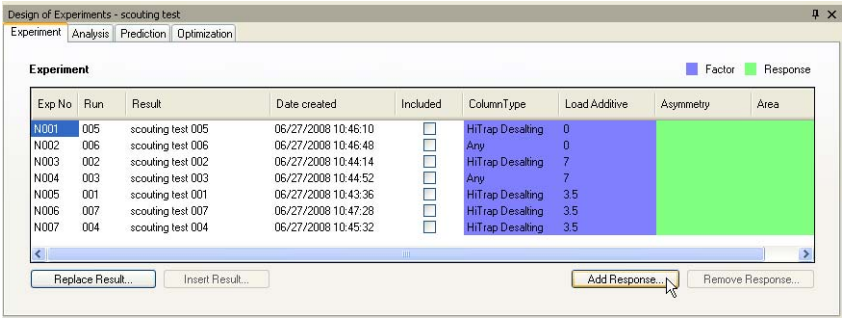
The current result contains two chromatograms. The chromatograms are displayed by clicking the corresponding tab. A ***Peak Table*** is also displayed under this chromatogram. Peak Tables are described in detail in *Section 4.2.3 Display peak data, on page 104*.

Docking chromatogram panes

If a result contains several chromatograms, each chromatogram will be displayed in its own pane. The chromatograms can be stacked on top of each other and selected by clicking the corresponding tab. They can also be arranged in various positions using a docking function. The arrangement of chromatograms is described in *Section 4.5.1 Open and compare chromatograms, on page 160*.

Design of Experiment results

The illustration below shows the ***Design of Experiment*** pane in the ***Evaluation*** module.



Design of Experiment results and the analysis, prediction and optimization of experiments are described in the *"UNICORN 6 Method Manual"*.

3 View and present the results

Introduction

This chapter describes how to present the chromatograms and curves of your result file and how to create and print reports.

In this chapter

This chapter contains these sections:

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3.2 Locate results	27
3.3 Optimize the presentation of chromatograms	36
3.4 Print chromatograms and peak data	57
3.5 Run documentation	60
3.6 Generate reports	70

3.1 Open and view results

Introduction

All contents of the result files are opened in the ***Evaluation*** module where you can analyze the results, create edited curves and compile reports. The ***Evaluation*** module user interface and toolbar icons are described in *Section 2.1 Evaluation module, on page 12*.

The result files that are located in folders accessible to you are shown in the ***Result Navigator***. There are different ways to locate the specific result that you are looking for. *Section 3.2 Locate results, on page 27* describes this. However, in this section an additional aid to identify a certain result and locate specific curves is also described. This preview function is called ***Quick View***. This section also describes how to highlight curves in a chromatogram, read curve values using a marker and save curve data as a ***Snapshot***.

How to open specific chromatograms and curves is described in *Section 4.5.1 Open and compare chromatograms, on page 160* and *Section 4.5.2 Open and compare curves, on page 167*.

Open a result in the Evaluation module

There are four ways to open a result file from the ***Result Navigator***:

- Select a result and click the **Enter** key

  or

- Double-click a result file

  or

- Right-click a result file and choose ***Open*** from the shortcut menu

  or

- Select a result file and click the ***Open*** toolbar icon in the ***Result Navigator***.

Result: The result is opened in the chromatogram pane.

Only one result at a time may be opened this way. If you open a new result, the previous result will automatically close. However, you may open several chromatograms from different results. This is described in *Section 4.5.1 Open and compare chromatograms, on page 160*.

Note: *When working in a network environment, it is not possible to edit the same result file from two different locations simultaneously. If the result is already open at another network workstation, you can only open it in read-only mode. A message similar to the illustration below will open.*

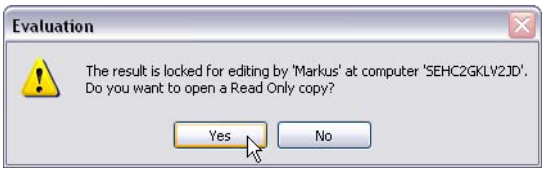
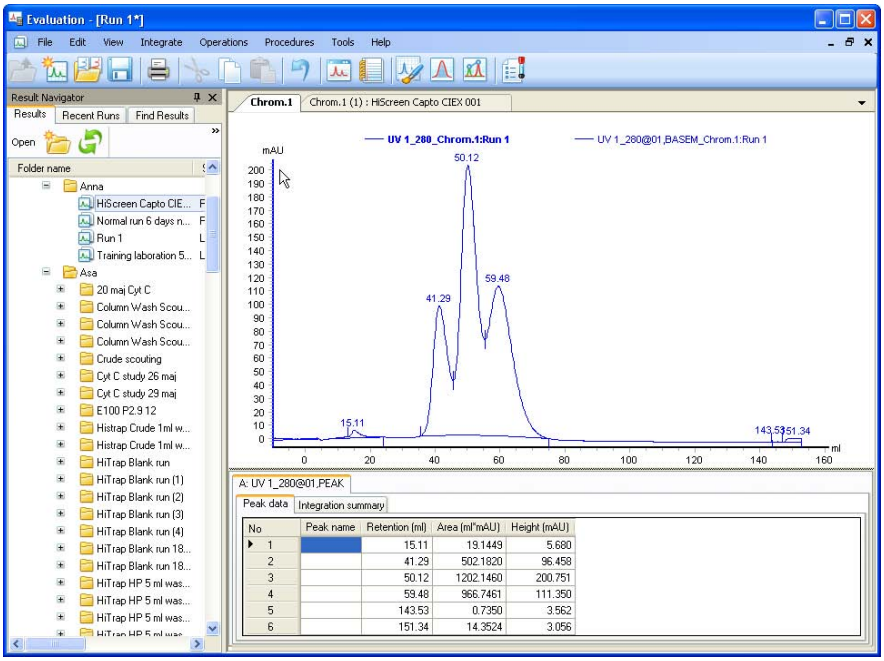

Evaluation
The result is locked for editing by 'Markus' at computer 'SEHC2GKLV2JD'.
Do you want to open a Read Only copy?
Yes
No

Illustration – a result open in the Evaluation module



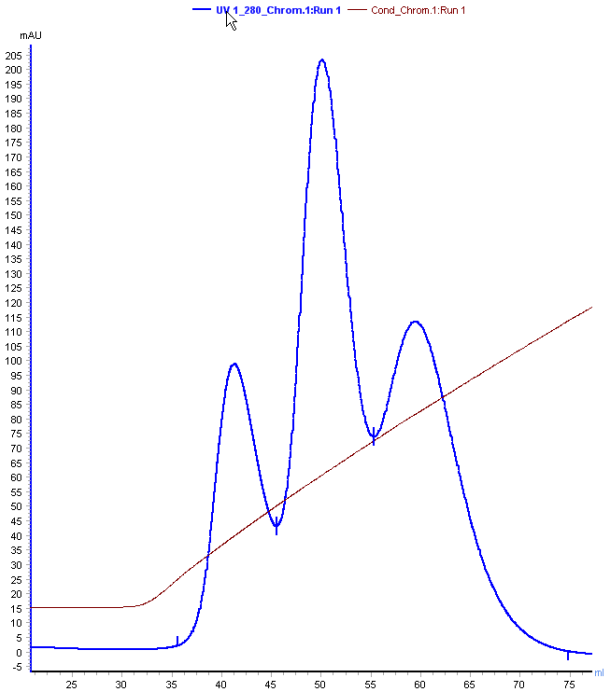
A result may contain several chromatograms and corresponding peak tables, which will open as one or several tabs. Each chromatogram can be selected by clicking the corresponding tab. The selected tab has a light color and its information is displayed in the active window.

The chromatograms may also be shown simultaneously if you choose the menu command ***View:Tile All Chromatograms***.

Highlight or select a curve

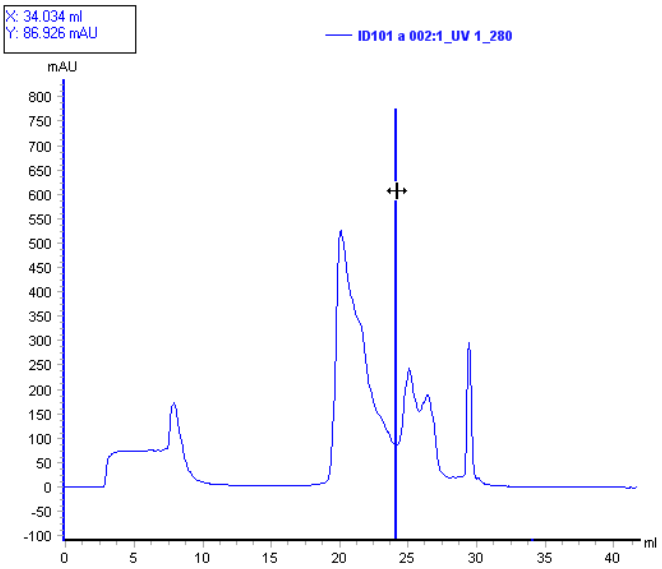
You can highlight or select an individual curve in the chromatogram. The table below describes the differences:

If you...	Then...
hold your mouse pointer over a curve segment	a pop-up box will display the curve name.
hold your mouse pointer over a curve name	the curve and the short line segment in front of the curve name become bold.
click a curve segment or a curve name	the curve name becomes bold and the Y-axis shows the values for this specific curve.



Insert a vertical marker

Right-click in the chromatogram and choose ***Vertical marker***. Move the marker along the X-axis and read the X-axis and Y-axis values of the selected curve in the box in the top left corner of the chromatogram.

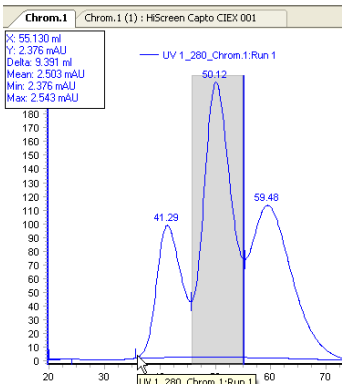
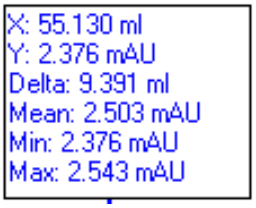


Note: *The marker will measure the curve that currently is selected if several curves are displayed in the chromatogram. The marker will have the same color as the selected curve.*

Set marker reference

You can use the vertical marker for more measurements than just the readings from a specific curve position. The table below describes how to use the marker to determine ***Delta Peak*** and ***Mean*** Y-axis values.

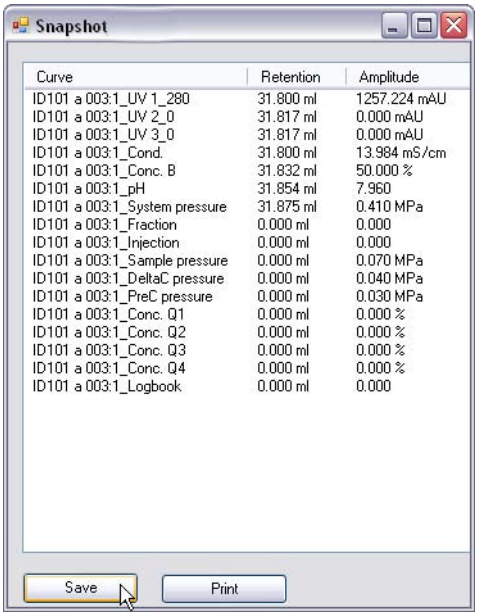
Step	Action
1	• Position the marker where you want to begin the measurement. • Right-click and choose ***Set vertical marker reference point***. *Result*: The reference point is set to the position shown in the box in the top left corner of the chromatogram.

Step	Action
2	• Drag the marker to the point where you want the measurement to end. *Result*: The measured area is colored as illustrated below: 
3	• Read the ***Delta*** and ***Mean*** values from the box: 

Snapshots

You can take a ***Snapshot*** of all the curve values at the marker position. The table below describes this:

Step	Action
1	• Position the marker where you want to take the ***Snapshot***. • Right-click and choose ***Snapshot*** from the shortcut menu. *Result*: The ***Snapshot*** dialog opens.



2	• Click the ***Save*** button to save the ***Snapshot*** *Result*: The ***Save As*** dialog opens and you can save the ***Snapshot*** as a text file. or • Click the ***Print*** button to print the ***Snapshot*** *Result*: The ***Print*** dialog opens and you can print the ***Snapshot*** using the selected printer.

Note: *Snapshots taken during the method run are saved directly to the result and can be accessed by clicking the* ***Snapshot*** *sub-tab in the* ***Result Information*** *tab of the* ***Documentation*** *dialog.*

Step	Action
3	Click the ***Close*** button to close the ***Snapshot*** dialog.

Note: *The snaphot will record only the values of curves that are displayed. Curves that are filtered will not be recorded.*

3.2 Locate results

Introduction

With exception for the ***Administration*** module, there are navigator panes available in all UNICORN modules. The navigator panes can be used to locate and open methods and result files and to arrange files in folders.

The ***Result Navigator*** in the ***Evaluation*** module consists of the same type of navigator pane as the other UNICORN modules, but also contains a pane for ***Recent Runs*** and a specific search pane, ***Find Results***. If desired, the ***Result Navigator*** can also display methods.

Show the navigator pane

To open and display the navigator panes, either

- choose the ***Result Navigator*** menu command in the ***View*** menu

  or

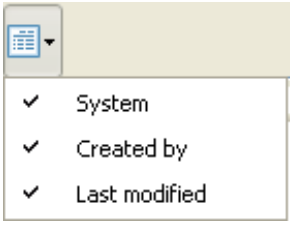
- click the ***Open Result Navigator*** toolbar icon.

Note: *As described in the general overview of the **Evaluation** module in Section 2.1 Evaluation module, on page 12, Auto Hide can be selected for the navigator panes.*

Navigator toolbar

The table below shows the navigator toolbar icons:

Icon	Function
	Opens the selected result. ***Note:*** *Selected items can also be opened by selecting the **File: Open** menu command or by double-clicking the file in the navigator pane. The **Open** command from the right-click shortcut menu can also be used to open selected chromatograms, curves or peak tables.*

Icon	Function
	Creates a new folder in the folder that is currently selected. ***Note:*** *You can also choose this function by selecting the **File:New Folder** menu command or selecting the **New Folder** command from the right-click shortcut menu.*
	Performs a ***Refresh*** in the navigator pane to update all items to the current status. ***Note:*** *You can also choose this function from the **View** menu. However, note that the **Refresh** menu command will update both the items in the navigator pane and items open in the module. The navigator toolbar icon will only update the navigator pane.*
	Opens the ***View Details*** droplist where the following optional information columns may be selected for display in the navigator pane: • System • Created by • Last modified date 

Filter settings

The ***Result Navigator*** will normally show only results and Design of Experiments results. This is the default filter setting for the items that are displayed. However, the navigator may also show items that primarily are used in another UNICORN module, i.e., methods and method queues. The options are:

- ***Results, DoE Results*** – showing only items that are relevant in the ***Evaluation*** module
- ***All*** – showing all items including method items and archived items
- ***All except Archived*** – only archived items are filtered

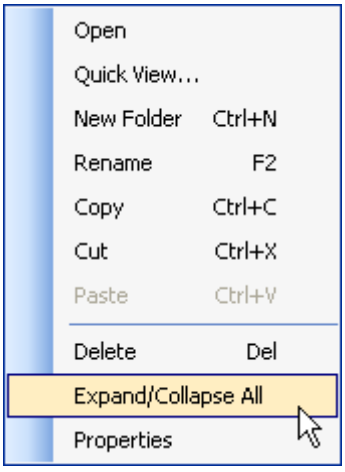
Note: *The navigator panes will only show folders that you have access to. Your access rights to folders are defined in the **User Setup** in the **Administration** module. The user setup process is described in the UNICORN Administration and Technical manual.*

Expand and collapse folders

When you select a folder in the navigators, the folders and results immediately below the selected folder are displayed.

If you have expanded the folder structure to lower levels, you may want to collapse all folders below a selected top folder, to simplify browsing.

- Right-click a folder and choose the ***Expand/Collapse All*** shortcut.



Result: The folder structure is collapsed to the selected folder level.

File properties

You can display some of the file properties of a selected result in the navigator pane.

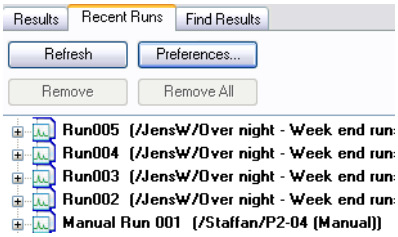
- Right-click a file and choose the ***Properties*** shortcut.

Result: The ***Properties*** dialog opens.

4.6 fractionation ID51 021 Properties
General
Result
Name:
4.6 fractionation ID51 021
Created by:
Roger
Created:
09/26/2008 15:45:33
Last Modified by:
Roger
Last Modified:
10/16/2008 13:38:31
Close

The Recent Runs pane

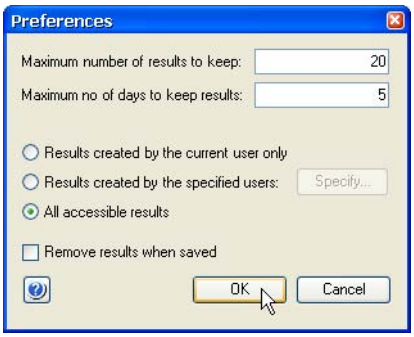
The ***Recent Runs*** pane shows all the available recorded recent runs based on the selected user preferences. The table below describes how to use ***Recent Runs*** to locate and open a result.

Step	Action
1	Click the ***Recent Runs*** tab in the ***Result Navigator***. *Result*: The ***Recent Runs*** pane opens.  ***Note:*** *Until the files and chromatograms in the list have been opened and saved manually, they are noted in bold text. When they are opened and saved the text is changed to plain text.*
2	If needed, click the ***Refresh*** button. *Result*: The ***Recent Runs*** list is updated with all runs that were performed since the ***Result Navigator*** was opened the last time.
3	Locate and double-click the desired result. *Result*: The result opens in the ***Evaluation*** module.

Note: *Click the **+** signs to view or select individual chromatograms from the results. Individual results can be selected and removed from the list by clicking the **Remove** button. The **Remove all** button clears the whole list. The **Remove** and **Remove all** buttons only clear the list, the files are not deleted.*

Recent Runs preferences

The ***Result Navigator*** will display ***Recent Runs*** based on the individual user preference settings. The table below describes how to adjust the preference settings:

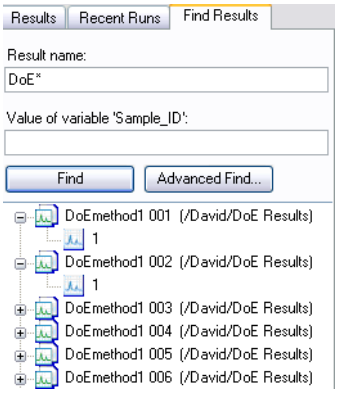
Step	Action
1	Click the ***Preferences*** button. *Result*: The ***Preferences*** dialog box opens. 
2	• Type the maximum number of results to keep on the list. • Type the maximum number of days to keep the results on the list.
3	Select which results to display on the list: • Only results created by the current user. • All results created by specified users. ***Note:*** *Click the **Specify** button to open a dialog box and select from a list with all accessible users.* • All accessible results regardless of the creator.
4	• Choose ***Remove results when saved*** if the results are to be removed from the list when they have been saved. • Click the ***OK*** button. *Result*: All new results will be displayed on the ***Recent Runs*** list based on the changed preferences.

Search for results using Find Results

The ***Find Results*** function in the ***Result Navigator*** is used to locate results in the available folders.

Note: *There is also a common **Find** function which is available in the **Edit** menu of all modules except the **Administration** module.*

The table below describes how to use the ***Find*** function to locate and open a result.

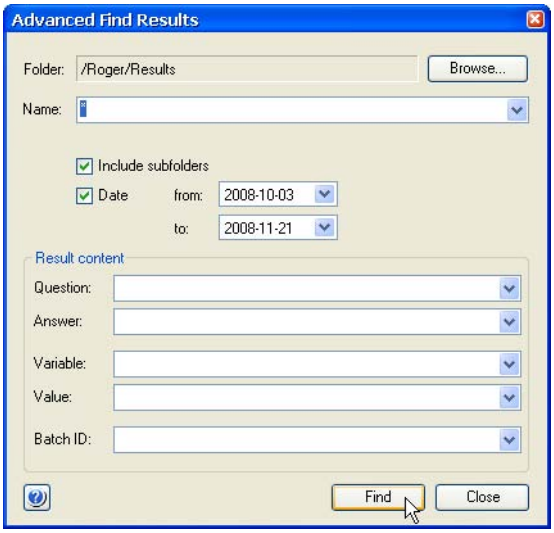
Step	Action
1	• Click the ***Find Results*** tab *Result*: The ***Find Results*** pane opens.
2	• Type a result name or part of a result name in the ***Result name*** text box. ***Note:*** *The standard wildcard character * can be used to represent a number of characters before or after the partial result name.* or • Type a Sample ID value in the ***Value of variable Sample_ID*** text box. ***Note:*** *A Sample ID can be set in the **Start Protocol**. The defined variable name must begin with **Sample_ ID**.*
3	• Click the ***Find*** button *Result*: The located results are listed. 
4	• Double-click the desired result or chromatogram *Result*: The file or chromatogram opens in the ***Evaluation*** module.

Note: *Click the **Advanced Find...** button for more advanced search functions, which are described below.*

Search for result files using Advanced Find Results

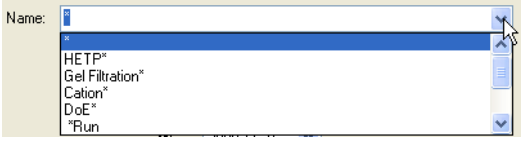
The table below describes how to use the ***Advanced Find Results*** function to locate and open a result file.

Step	Action
1	• Click the ***Advanced Find...*** button *Result*: The ***Advanced Find Results*** dialog opens.
2	• Click the ***Browse*** button to display the folder structure in a ***Browse Folder*** dialog, and select a top folder to search in. ***Note:*** *Your home folder will be selected by default.* • Type a file name or part of a file name in the ***Result name*** text box. ***Note:*** *The standard wildcard character * can be used to represent a number of characters before or after the partial file name.*



Step	Action
3	• Select if the search shall ***Include subfolders***. • Select if the search shall be limited to a range of creation dates.
4	If desired, specify ***Result content*** to search for results containing: • ***Questions*** (as defined in the ***Start Protocol***) • ***Answers*** (as defined in the ***Start Protocol***) • ***Variable*** (specific names) • ***Value*** (specific variable values) • ***Batch ID*** (specific values) *Result*: The file or chromatogram opens in the ***Evaluation*** module.
5	• Click the ***Find*** button. *Result*: The located files are listed in the ***Find Results*** pane. • Click the ***Close*** button to close the ***Advanced Find Results*** dialog.

Note: *Previous search qualifiers can be found in the droplists by each text box. The qualifiers are listed in reverse order with the latest qualifier on top:*



Close the navigator pane

To close the navigator pane:

- Click the small cross in the top right-hand corner of the navigator.

Result: The navigator closes.

3.3 Optimize the presentation of chromatograms

Introduction

This section describes some of the ways you can optimize the presentation of a chromatogram.

In this section

This section contains these sub-sections:

Section	See page
3.3.1 Customize the chromatogram layout	37
3.3.2 Edit curve presentation	42
3.3.3 Change the axes	47
3.3.4 Enter and edit text in chromatograms	51
3.3.5 Save and apply layouts	53

3.3.1 Customize the chromatogram layout

Introduction

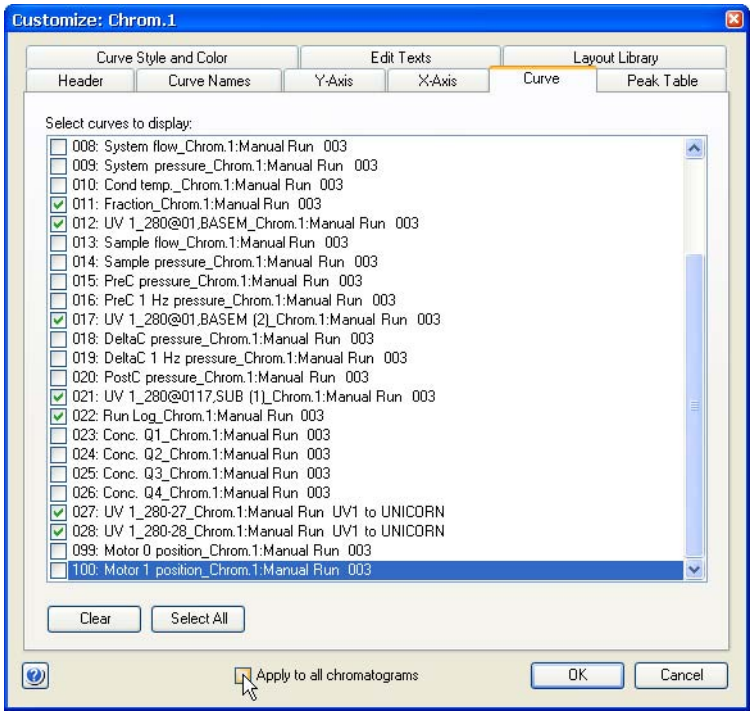
The ***Customize*** dialog box is used to make changes regarding chromatogram presentation. The main features of the ***Customize*** dialog box regarding chromatograms are described in the subsequent sections in this chapter. Features regarding peak tables are described in *Section 4.2 Peak integration, on page 98*.

Instruction

The table below describes how to make changes in the ***Customize*** dialog box:

Step	Action
1	Open a result file.
2	• Right-click the chromatogram and select ***Customize*** or • Choose ***Tools:Customize*** or • Click the ***Customize*** toolbar icon. *Result:* The ***Customize*** dialog box is displayed.

Step	Action
3	• Carry out the changes on the different tabs to get the desired layout for header, curves and peak table.


Customize: Chrom.1
Curve Style and Color
Edit Texts
Layout Library
Header
Curve Names
Y-Axis
X-Axis
Curve
Peak Table
Select curves to display:
008: System flow_Chrom.1:Manual Run 003
009: System pressure_Chrom.1:Manual Run 003
010: Cond temp._Chrom.1:Manual Run 003
011: Fraction_Chrom.1:Manual Run 003
012: UV 1_280@01,BASEM_Chrom.1:Manual Run 003
013: Sample flow_Chrom.1:Manual Run 003
014: Sample pressure_Chrom.1:Manual Run 003
015: PreC pressure_Chrom.1:Manual Run 003
016: PreC 1 Hz pressure_Chrom.1:Manual Run 003
017: UV 1_280@01,BASEM (2)_Chrom.1:Manual Run 003
018: DeltaC pressure_Chrom.1:Manual Run 003
019: DeltaC 1 Hz pressure_Chrom.1:Manual Run 003
020: PostC pressure_Chrom.1:Manual Run 003
021: UV 1_280@0117,SUB (1)_Chrom.1:Manual Run 003
022: Run Log_Chrom.1:Manual Run 003
023: Conc. Q1_Chrom.1:Manual Run 003
024: Conc. Q2_Chrom.1:Manual Run 003
025: Conc. Q3_Chrom.1:Manual Run 003
026: Conc. Q4_Chrom.1:Manual Run 003
027: UV 1_280-27_Chrom.1:Manual Run UV1 to UNICORN
028: UV 1_280-28_Chrom.1:Manual Run UV1 to UNICORN
099: Motor 0 position_Chrom.1:Manual Run 003
100: Motor 1 position_Chrom.1:Manual Run 003
Clear
Select All
Apply to all chromatograms
OK
Cancel

	• Select ***Apply to all chromatograms*** if you want to apply changes made in the ***Customize*** dialog to all open chromatograms.
4	Click ***OK*** to apply the changes.

Tabs in the Customize dialog

The table below lists the tabs of the ***Customize*** dialog and outlines the editing functions of each tab.

Tab	Use this tab to...
Header	select the items to be displayed in the header field above the curves: • Scouting variables • Variables • Questions • Notes
Curve Names	select how the curve names are displayed. This is described in detail in *Section 3.3.2 Edit curve presentation, on page 42*.
Y-axis	determine how the Y-axis is displayed. This is described in detail in *Section 3.3.3 Change the axes, on page 47*.
X-axis	determine how the X-axis is displayed. This is described in detail in *Section 3.3.3 Change the axes, on page 47*.
Curve	select the curves to display in the chromatogram.
Peak Table	select the ***Peak Table*** settings. This is described in detail in *Section 4.2.3 Display peak data, on page 104*.
Curve Style and Color	determine how the curves are displayed. This is described in detail in *Section 3.3.2 Edit curve presentation, on page 42*.
Edit Texts	edit any text that has been added to the chromatogram. This is described in detail in *Section 3.3.4 Enter and edit text in chromatograms, on page 51*.

Tab	Use this tab to...
Layout Library	• select and apply a saved chromatogram layout or • delete a saved layout or • save the current layout. This is described in detail in *Section 3.3.5 Save and apply layouts, on page 53*.

Note: *You can select to apply the settings in all tabs to all open chromatograms, with the exception of text. Text entries can only be applied to the current chromatogram.*

Layout options in the right-click menu

You can also change the chromatogram layout by choosing some menu items from the right-click shortcut menu. The table below describes the layout options available on this menu:

Choose...	when you want to...
Grid	add a grid to the background of the chromatogram.
Filter Curves	add a list of all available curves to the chromatogram, with checkboxes to select the curves to display: ***Note:*** *Click the arrow buttons to show additional curves that do not fit in the available space.*

Choose...	when you want to...
Select Active Curve Only	show only the active curve in the chromatogram. (this option is available only when ***Filter Curves*** is selected)
Select All Curves	show all curves in the chromatogram. (this option is available only when ***Filter Curves*** is selected)
Legend	show the list of all displayed curve names over the curve area of the chromatogram.
• ***Time*** or • ***Volume*** or • ***Column volume***	select the unit of the X-axis.

3.3.2 Edit curve presentation

Select curves to display

The ***Curve*** tab of the ***Customize*** dialog contains a list of all the curves included in the chromatogram. Select the curves you want to display in the chromatogram, and click ***OK***.

Curve name appearance

You select options for the curve name appearance on the ***Curve Names*** tab. This is an example of a default curve name:

ID101 a001:1 UV 1_280

The table below describes the three components that make up the default curve name:

Component	Description	Example
Result name	Name of the result. This is defined by the settings in the ***Result Name & Location*** dialog in the ***Method Editor***.	ID001 a 001
Chromatogram name	Number given automatically during a run.	1
Curve name	Curve type, for example a monitor signal.	UV 1_280

Note: *A name may also be defined in the* ***New Chromatogram*** *text instruction.*

Choose curve name appearance

You can choose to view only part of the curve name. The table below describes how to do this:

Step	Action
1	Open the ***Customize*** dialog.
2	Click the ***Curve Names*** tab.
3	Select the appropriate boxes for ***Curve name appearance***.
4	Click ***OK***.

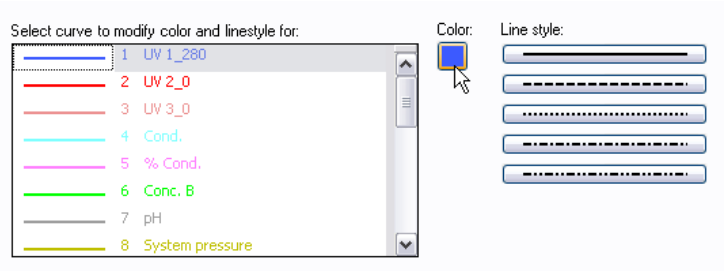
Note: *It is usually sufficient to select the **Curve Name** option if only one chromatogram is being evaluated. However, confusion can arise when more than one chromatogram is shown, so more complete names might be necessary.*

Curve style and color settings

All curves within a chromatogram are represented by a default color and line style. Curves that are imported into the chromatogram or newly created curves are automatically assigned a color and line style. The settings are available in the ***Curve Style and Color*** tab. The tab also contains settings for peak labels, fraction marks and run log texts.

Change the color and style of a curve

The table below describes how to change the color and style of a curve:

Step	Action
1	Open the ***Customize*** dialog.
2	Click the ***Curve Style and Color*** tab.
3	• Select the curve you want to change from the list. • Click the ***Color*** button to open the ***Color*** dialog and choose a color from the palette. • Click a button to choose a ***Line style***.  • Repeat step 3 to modify other curves.
4	Click the ***OK*** button to apply the changes.

Peak labels

Peaks can be labelled on the ***Curve Style and Color*** tab. A combination of the following labels can be selected:

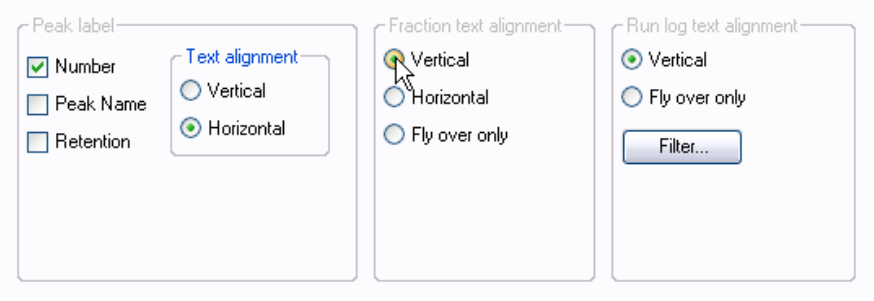
- ***Number***
- ***Peak Name***
- ***Retention*** (the default label).

You can also select ***Vertical*** or ***Horizontal*** alignment for the text.

Note: *You will need to perform a peak integration and create a peak table before the labels are displayed. The peak name is entered in the peak table. This is described in* *Section 4.2.4 Edit the integration parameters, on page 108*.

Align Fraction and Run log texts

Both ***Fraction*** and ***Run log*** text can be set either to be displayed when the mouse pointer is positioned over a fraction mark (***Fly over only***) or at all times. The ***Fraction*** text can be aligned both vertically and horizontally. Default setting for both text types is ***Vertical***. The ***Vertical*** selection for ***Run log*** text is the setting to show the text at all times.



Filter Run log information

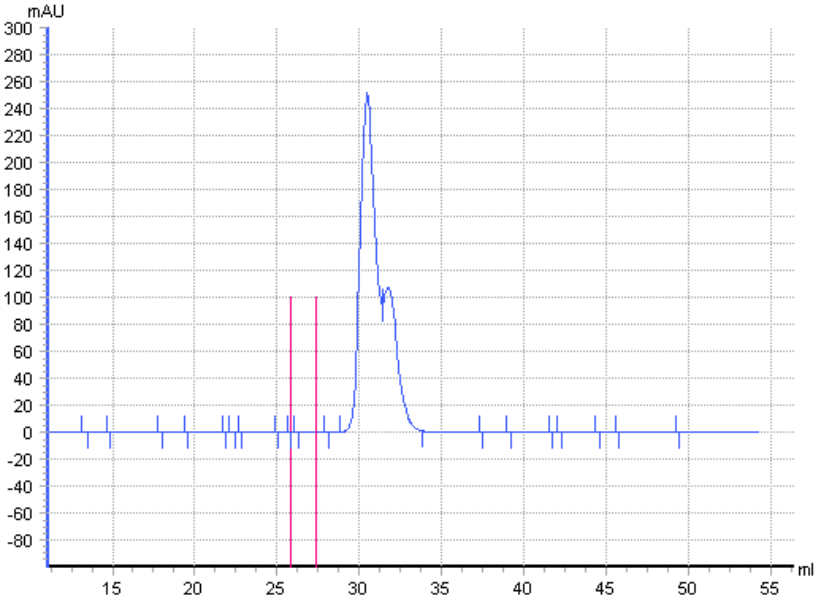
The table below describes how to select the Run log entry and feedback types to show in the chromatogram:

Step	Action
1	Open the ***Customize*** dialog.
2	Click the ***Curve Style and Color*** tab.

Step	Action
3	Click the ***Filter*** button in the ***Run log text alignment*** field: *Result*: The ***Filter Run log*** dialog opens.
4	• Select the entry types to show in the chromatogram. • Select the feedback types to include in the run log entries. • Click the ***OK*** button. *Result*: The ***Filter Run log*** dialog closes.
5	Click the ***OK*** button in the ***Customize*** dialog to apply the changes.

Add a grid

You can add a grid to the background of the chromatogram by clicking the ***Grid*** checkbox.



Note: *You can also right-click in the chromatogram and choose **Grid** from the shortcut menu.*

3.3.3 Change the axes

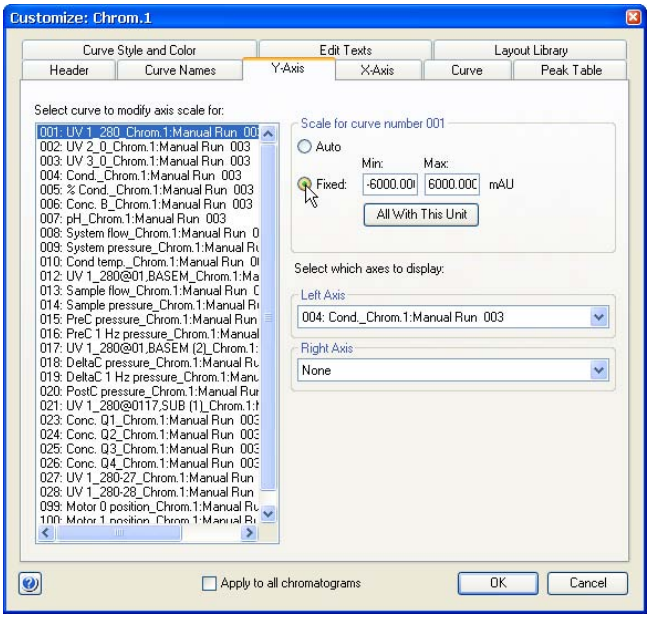
Introduction

This section describes how to change the chromatogram axes according to your preferences. These changes are made in the Y-axis and X-axis tabs of the Customize dialog.

Change and fix the Y-axis

The table below describes how to change and fix the scale of the curves in the chromatogram Y-axis:

Step	Action
1	Click the ***Y-Axis*** tab.
2	• Select the appropriate curve from the list. • Click the ***Fixed*** option in the ***Scale for curve number...*** field.



Step	Action
3	• Type the desired minimum and maximum values. • Click the ***All with this unit*** button if you want other curves with the same Y-axis units as the current scaled curve to be similarly scaled. ***Note:*** *The values will only be applied to existing curves. They will not be applied to new curves created after this function was last used.*
4	Click ***OK*** to apply the changes.

Select Y-axes to display

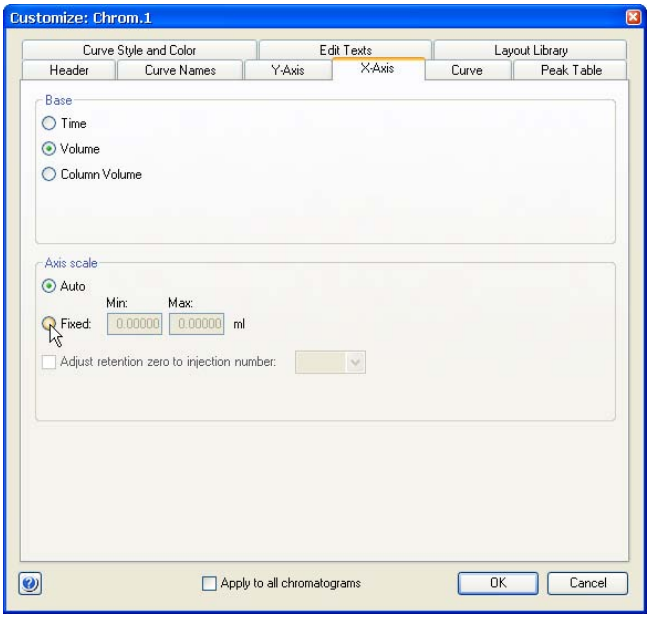
The table below describes how to select the Y-axes to display in the chromatogram.

Step	Action
1	Click the ***Y-Axis*** tab.
2	• Select a curve for the left Y-axis in the ***Left Axis*** droplist. • If you want to add a second Y-axis, proceed with step 3. If not, jump to step 4.
3	Select a second curve from the ***Right Axis*** droplist.
4	Click ***OK*** to apply the changes.

Change and fix the X-axis

The table below describes how to change and fix the scale of the X-axis in the ***Customize*** dialog:

Step	Action
1	Click the ***X-Axis*** tab.



2	Select the appropriate option in the ***Base*** field: • ***Time*** • ***Volume*** • ***Column Volume***

Note: *Some calculated curves, for example baselines, exist in only one base and might seem to disappear when the base is changed. Curves are collected in time and recalculated for display in volume. Thus, switching the base between* ***Time*** *and* ***Volume*** *can slightly alter the resolution of the displayed curves.*

Step	Action
3	• Click the ***Fixed*** option in the ***Axis scale*** field to set the axis limits manually. • Type the desired minimum and maximum values. • If desired, select the ***Adjust retention zero to injection number*** checkbox and choose an injection number from the droplist. ***Note:*** *This sets the time/volume to zero at the selected injection mark. The time and volume before selected injection will become negative values.*
4	Click ***OK*** to apply the changes.

3.3.4 Enter and edit text in chromatograms

Introduction

This section describes how to enter and edit text annotations in the curve field of the chromatogram. The text is applicable to the active chromatogram.

Enter annotation text

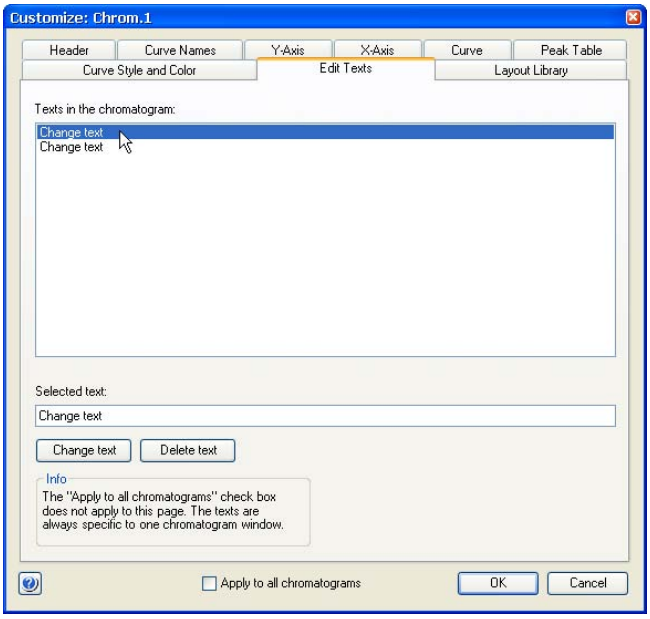
The table below describes how to enter annotation text in the chromatogram:

Step	Action
1	• Right-click the curves view of the chromatogram window and select ***Add text*** from the menu. or • Choose ***Edit:Text:Add***.
2	• Click where you want to insert text in the chromatogram. *Result*: A text box opens.
3	• Double-click inside the text box. • Type the new text. • Click outside the text box to set the text. ***Note:*** *You can select and drag the text box to a new position with your mouse pointer.*

Edit annotation text

The table below describes how to edit inserted annotation text:

Step	Action
1	Choose ***Edit:Text:Edit***. *Result:* The ***Edit Texts*** tab of the ***Customize*** dialog is displayed.



2	• Select the text that you want to edit and make the appropriate changes in the ***Selected text*** field. • Click the ***Change text*** button or the ***Delete text*** button.
3	Click **OK** to apply the changes.

Shortcut option

You can also double-click inside the text box to select the text. Then you can

- type new text

  and
- click outside the text box to set the new text

3.3.5 Save and apply layouts

Introduction

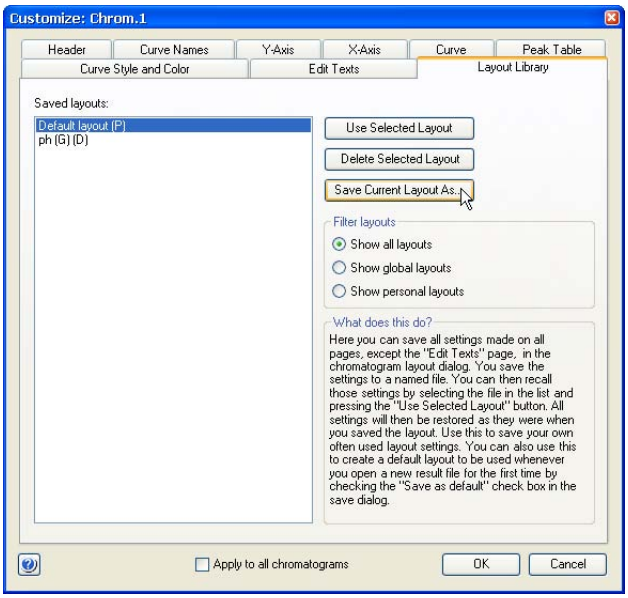
All configurations that you make in the ***Customize*** dialog can be saved as a layout. It is possible to apply saved layouts to other chromatograms. Layouts can either be saved as personal layouts for your own use only, or as global layouts that are available for everybody with network access. You may also save a layout as your default setting. The default layout will be applied automatically when you open a result.

Save a layout

The table below describes how to save a layout:

Step	Action
1	Open the ***Customize*** dialog.
2	• Make the appropriate layout configuration within the various tabs. • Click ***OK*** to see the applied affects of your changes. • Return to the ***Customize*** dialog box to perform further changes.

Step	Action
3	Select the ***Layout Library*** tab when you are satisfied with the layout.

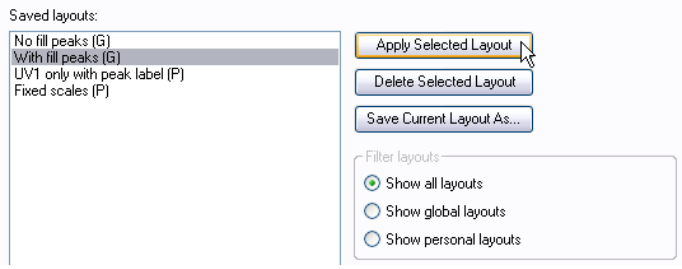

Customize: Chrom.1
Header
Curve Names
Y-Axis
X-Axis
Curve
Peak Table
Curve Style and Color
Edit Texts
Layout Library
Saved layouts:
Default layout (P)
ph (G) (D)
Use Selected Layout
Delete Selected Layout
Save Current Layout As...
Filter layouts
Show all layouts
Show global layouts
Show personal layouts
What does this do?
Here you can save all settings made on all pages, except the "Edit Texts" page, in the chromatogram layout dialog. You save the settings to a named file. You can then recall those settings by selecting the file in the list and pressing the "Use Selected Layout" button. All settings will then be restored as they were when you saved the layout. Use this to save your own often used layout settings. You can also use this to create a default layout to be used whenever you open a new result file for the first time by checking the "Save as default" check box in the save dialog.
Apply to all chromatograms
OK
Cancel

4	Click the ***Save Current Layout As*** button. *Result*: The ***Save Layout*** dialog box is displayed.

Step	Action
5	• Type a name for the layout in the ***Save layout as:*** textbox. • Select if the layout should be ***Global*** or ***Personal***. • If you want the current layout to be the new default layout, select the ***Save as default*** checkbox. **TIP:** The default layout will be applied automatically when you open a result.
6	Click ***OK*** to save the layout and close the ***Save Layout*** dialog. *Result:* The new layout name is added to the ***Saved layouts*** list.
7	Click ***OK*** to close the ***Customize*** dialog.

Apply a layout

The table below describes how to apply a saved layout:

Step	Action
1	Select the ***Layout Library*** tab on the **Customize** dialog.
2	• Select a layout from the ***Saved layouts*** list.  • If necessary, select which layouts to show in the ***Filter layouts*** field. ***Note:*** *Personal layouts are noted with a **(P)**, global layouts with a **(G)**.* • Click the ***Apply selected layout*** button.
3	If the same layout is to be applied to all chromatograms on the ***Evaluation*** workspace, select the ***Apply to all chromatograms*** checkbox.

Step	Action
4	Click ***OK*** to close the dialog and apply the changes.

3.4 Print chromatograms and peak data

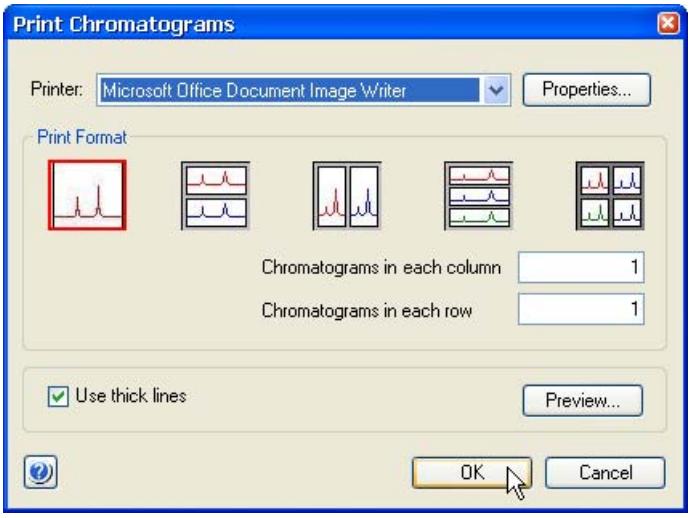
Introduction

This section describes how to print the chromatograms that are open in the ***Evaluation*** module.

The Print Chromatograms dialog box

This is an illustration of the ***Print Chromatograms*** dialog.

Note: *The selected print format is outlined in red.*

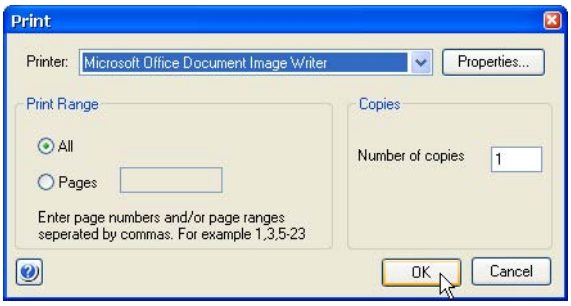


Instruction

The table below describes how to print active chromatograms on the default Windows printer.

Step	Action
1	Open all chromatograms that you want to print in the ***Evaluation*** module.

Step	Action
2	• Select ***File:Print***. or • Click the ***Print result*** toolbar icon. *Result*: The ***Print Chromatograms*** dialog opens.
3	Select print format and layout options.
4	• Click ***OK*** to open the ***Print*** dialog and proceed with step 8. or • Proceed with step 5 to preview and edit the layout.
5	Click the ***Preview*** button. *Result*: The ***Customize Report*** window opens.
6	• Click the ***Edit Mode*** button to make changes, for example to change the order of the chromatograms (see *Section 3.6.2 Create a new report format, on page 74* for more information about how to edit). • Click the ***Preview*** button to return to preview mode. ***Note:*** *You can print directly from the* ***Customize Report*** *window or click the* ***Exit*** *button to return to the* ***Print Chromatogram*** *dialog.*

Step	Action
7	• Select ***File:Print***. or • Click the ***Print*** toolbar icon. *Result*: The ***Print*** dialog opens. 
8	• Select the print range and number of copies. • If necessary, click the ***Properties*** button to open the printer properties dialog where the settings for your printer can be changed. • Click ***OK*** to print the chromatograms.

3.5 Run documentation

Introduction

The full documentation for a method run is stored in the result file. This section contains:

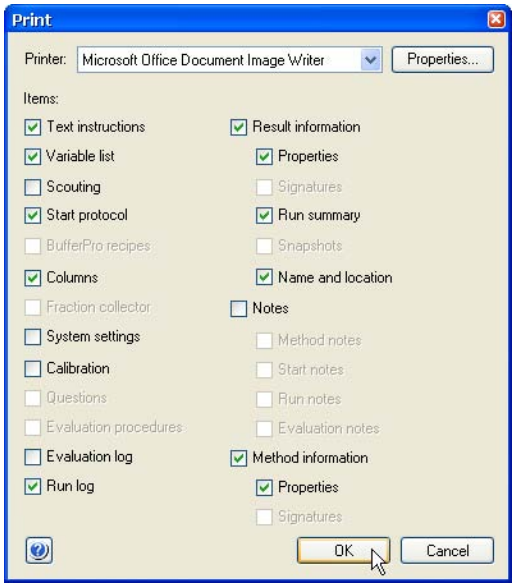
- an instruction how to view and print the run documentation,
- a list and short descriptions of the contents of the run documentation,
- an instruction how to save the text instructions from the run as a new method.

View and print the run documentation

The table below describes how to view and print the run documentation.

Step	Action
1	Open a result file in the ***Evaluation*** module.
2	• Choose ***View: Documentation*** or • Click the view ***Documentation*** icon. *Result*: The ***Documentation*** dialog opens. See further information about the tabs and contents below.
3	• Click the ***Print*** button. *Result*: The ***Print*** dialog opens.

Step	Action
4	• Select the documentation items you want to print. ***Note:*** *Items that do not contain any information cannot be selected. If you select a group heading (e.g., **Result information**) all sub-headings that contain information will automatically be selected.*



5	Click the ***OK*** button to print the selected items.

The Documentation tabs

The table below describes the contents of the **Run Documentation** tabs.

Note: *Some of the items listed below may be excluded from the **Run Documentation**. Tabs will be shown only for items that have been included in the method.*

Documentation tab	Contents
Result Information	The ***Result Information*** tab contains general information about the result. See *"The Result Information tab"* in this section.

Documentation tab	Contents
Evaluation Procedures	The ***Evaluation Procedures*** tab shows all procedures that have been run during or after the method run. See *Section 6.3 Automated evaluation procedures, on page 224* for information about how to work with evaluation procedures.
Method Information	The ***Method Information*** tab shows the general ***Properties*** of the method: • Name • When and by whom the method was created • When and by whom the method was last modified • The system and ***Instrument Configuration*** that the method was created for Click the ***Details*** button to view comprehensive information about the ***Instrument Configuration***. The ***Method Information*** tab also contains a listing of all the electronic signatures that have been added to the method, under the sub-tab ***Signatures***.
Start Protocol	The ***Start Protocol*** tab shows the method items that were included in the ***Start Protocol*** at the start of the method run.
System Settings	The ***System Settings*** tab shows the system settings during the method run, for example • UV, conductivity and pH settings • Pumps and pressure settings • Air sensor settings, watches and alarms • Fraction collection settings
Calibration	The ***Calibration*** tab shows a calibration report for the system.
Run Log	The ***Run Log*** tab shows selected log entries and feedback. See *"The Run Log and Evaluation Log tabs"* in this section.

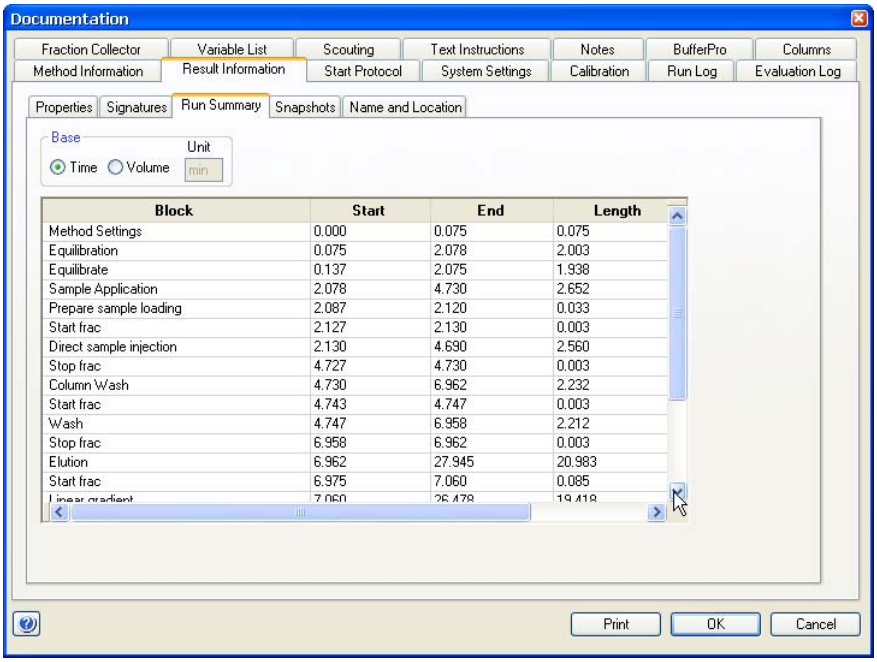
Documentation tab	Contents
Evaluation Log	The ***Evaluation Log*** tab lists all of the evaluation operations that have been performed for the result during all sessions, including at the end of the method run. The log also shows when the result has been accessed without editing after the end of the method run. See *"The Run Log and Evaluation Log tabs"* in this section.
Fraction Collector	The ***Fraction Collector*** tab shows an image of the cassettes, tubes and well plates used in the fraction collector.
Variable List	The ***Variable List*** tab shows all the method variables and corresponding values, listed by the method block where they appear. The list can show detail and/or unused variables. At this stage, the variable values are no longer possible to edit.
Scouting	The ***Scouting*** tab shows the scouting parameter settings. This tab corresponds to the ***Scouting*** settings in the ***Method Editor***.
Text Instructions	The ***Text Instructions*** tab shows all the text method instructions from the method. These instructions can be saved as a new method. This is described in *"Save the method used for the run as a new method"* in this section.
Notes	The ***Notes*** tab shows all notes that have been made regarding the method, the method run and the subsequent evaluation. The notes are divided into the following sub-tabs: • ***Method Notes*** • ***Start Notes*** • ***Run Notes*** • ***Evaluation Notes*** You can add new notes on the ***Evaluation Notes*** sub-tab. ***Note:*** *Click the **Find** button to search for specific text in the **Notes**.*

Documentation tab	Contents
BufferPro	The ***BufferPro*** tab shows the ***BufferPro*** settings, including the recipe settings, the stock solutions and a general description.
Columns	The ***Columns*** tab shows information about the columns used during the method run. It contains two sub-tabs: **Column type** The ***Column type*** tab shows general information about the type of column that has been used, including: • general ***Run Parameters*** • column ***Details*** (dimensions, volumes, bed heights, etc.) • ***Ordering Information*** **Column** The ***Column*** tab shows specific information for the column that has been used, including: • ID numbers • ***Media Batch ID*** and expiration date • number of runs the column has been used for • results from performance tests

The Result Information tab

The table below describes the sub-tabs and contents of the ***Result Information*** tab:

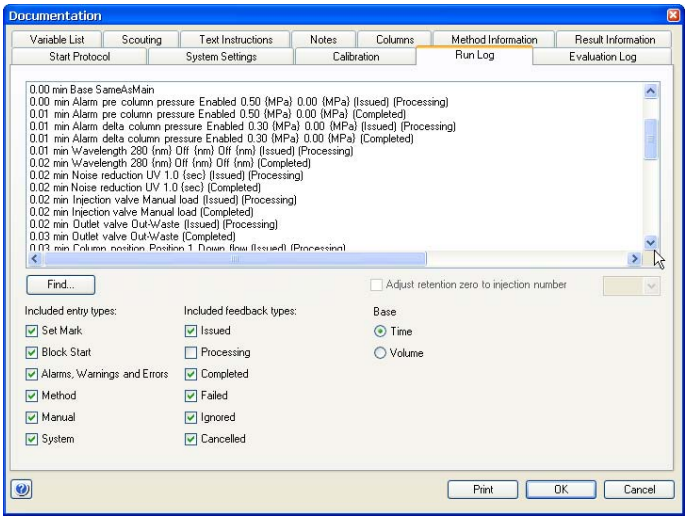
Result Information sub-tab	Contents
Properties	The ***Properties*** sub-tab shows general information about the result: • Name • Batch ID • When and by whom the result was created • When and by whom the result was last modified • The system and ***Instrument Configuration*** that was used for the method run Click the ***Used Components*** button to view a list of all components and monitors that were used for the run. Click the ***Details*** button to view comprehensive information about the ***Instrument Configuration***.
Signatures	The ***Signatures*** sub-tab contains a listing of all the electronic signatures that have been added to the result.
Run Summary	The ***Run Summary*** sub-tab (illustrated below) shows a summary of the run expressed in volume or time per phase and block. The phases are shown with all capital letters. Method blocks within the phases are shown as they appear in the ***Text Instructions***. The method ***Base*** can be toggled between ***Time*** and ***Volume*** by selecting the corresponding radio button.
Snapshots	The ***Snapshots*** sub-tab shows all snapshots that have been taken during the run.
Name and Location	The ***Name and Location*** sub-tab shows the search path to the result, as an image of the folder structure where the result is saved. This sub-tab corresponds to the ***Result Name and Location*** dialog in the ***Method Editor***.


Documentation
Fraction Collector
Variable List
Scouting
Text Instructions
Notes
BufferPro
Columns
Method Information
Result Information
Start Protocol
System Settings
Calibration
Run Log
Evaluation Log
Properties
Signatures
Run Summary
Snapshots
Name and Location
Base
Time
Volume
Unit
min
Block
Start
End
Length
Method Settings 0.000 0.075 0.075
Equilibration 0.075 2.078 2.003
Equilibrate 0.137 2.075 1.938
Sample Application 2.078 4.730 2.652
Prepare sample loading 2.087 2.120 0.033
Start frac 2.127 2.130 0.003
Direct sample injection 2.130 4.690 2.560
Stop frac 4.727 4.730 0.003
Column Wash 4.730 6.962 2.232
Start frac 4.743 4.747 0.003
Wash 4.747 6.958 2.212
Stop frac 6.958 6.962 0.003
Elution 6.962 27.945 20.983
Start frac 6.975 7.060 0.085
Print
OK
Cancel

The Run Log and Evaluation Log tabs

The ***Run Log*** and ***Evaluation Log*** are similar but cover different process cycles.

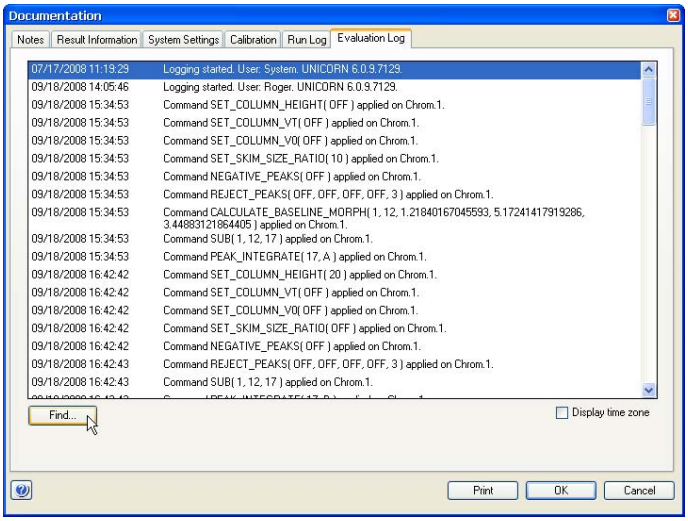
Run Log



The ***Run Log*** shows settings and events from the initial start of the method run up to the end of the run.

The displayed run log can be filtered to show only selected entry and feedback types. The ***Base*** can be shown either as ***Time*** or ***Volume*** and the retention zero can be adjusted to a selected injection.

Evaluation Log


Documentation
Notes
Result Information
System Settings
Calibration
Run Log
Evaluation Log
07/17/2008 11:19:29 Logging started. User: System. UNICORN 6.0.9.7129.
09/18/2008 14:05:46 Logging started. User: Roger. UNICORN 6.0.9.7129.
09/18/2008 15:34:53 Command SET_COLUMN_HEIGHT(OFF) applied on Chrom.1.
09/18/2008 15:34:53 Command SET_COLUMN_VT(OFF) applied on Chrom.1.
09/18/2008 15:34:53 Command SET_COLUMN_V0(OFF) applied on Chrom.1.
09/18/2008 15:34:53 Command SET_SKIM_SIZE_RATIO(10) applied on Chrom.1.
09/18/2008 15:34:53 Command NEGATIVE_PEAKS(OFF) applied on Chrom.1.
09/18/2008 15:34:53 Command REJECT_PEAKS(OFF, OFF, OFF, OFF, 3) applied on Chrom.1.
09/18/2008 15:34:53 Command CALCULATE_BASELINE_MORPH(1, 12, 1.21840167045593, 5.17241417919286, 3.44883121864405) applied on Chrom.1.
09/18/2008 15:34:53 Command SUB(1, 12, 17) applied on Chrom.1.
09/18/2008 15:34:53 Command PEAK_INTEGRATE(17, A) applied on Chrom.1.
09/18/2008 16:42:42 Command SET_COLUMN_HEIGHT(20) applied on Chrom.1.
09/18/2008 16:42:42 Command SET_COLUMN_VT(OFF) applied on Chrom.1.
09/18/2008 16:42:42 Command SET_COLUMN_V0(OFF) applied on Chrom.1.
09/18/2008 16:42:42 Command SET_SKIM_SIZE_RATIO(OFF) applied on Chrom.1.
09/18/2008 16:42:42 Command NEGATIVE_PEAKS(OFF) applied on Chrom.1.
09/18/2008 16:42:43 Command REJECT_PEAKS(OFF, OFF, OFF, OFF, 3) applied on Chrom.1.
09/18/2008 16:42:43 Command SUB(1, 12, 17) applied on Chrom.1.
Find...
Display time zone
Print
OK
Cancel

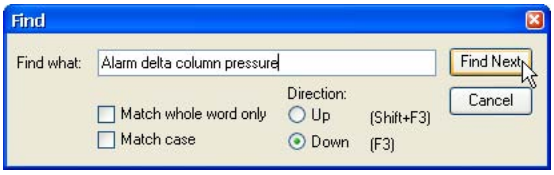
The ***Evaluation Log*** is initiated by the first evaluation action and additional entries are added every time the result is accessed.

The evaluation operations are listed in the order that they have been performed. If desired, the time zone can be added to each entry.

Search for log entries

The table below describes how to find specific text in the logs.

Step	Action
1	Select the log tab where you want to perform your search.
2	• Click the ***Find*** button. *Result*: The ***Find*** dialog opens.



Step	Action
3	• Type the text you want to locate in the ***Find what:*** textbox. ***Note:*** *Your previous search text may be shown in this box if you have used the search function before.* • Select additional search criteria: - ***Match whole word only*** - ***Match case*** - ***Search up*** - ***Search down***
4	• Click the ***Find Next*** button. *Result*: The first log entry where the search text is found is marked.

Save the method used for the run as a new method

You can save the method and the variables that were used for the run as a new method:

Step	Action
1	Select the ***Text Instructions*** tab.
2	• Click the ***Save as*** button. *Result*: The ***Save As*** dialog box opens.
3	• Select the appropriate destination folder. • Type a name in the ***Name*** text box. • Select a system from the ***System*** droplist.
4	• Click ***OK***. *Result*: The method is saved.

3.6 Generate reports

Introduction

The **Evaluation** module provides extensive tools to create detailed reports. This section describes how to create and print reports and how to customize the report layouts.

In this section

This section contains these topics.

Section	See page
3.6.1 Generate and print a predefined report format	71
3.6.2 Create a new report format	74
3.6.3 Edit an existing report format	91

3.6.1 Generate and print a predefined report format

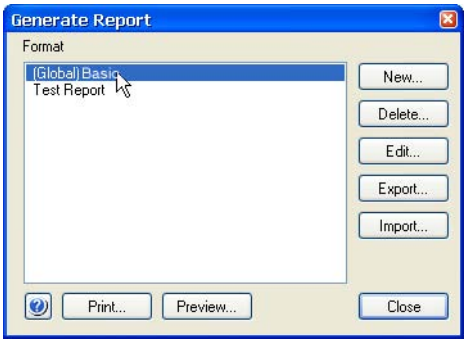
Introduction

This section describes how to generate and print a report using a format that has been defined and saved.

Should you need to store your reports in an electronic format you can also save them as PDF files. This section describes how to do this.

Generate and print the report

The table below describes how to select a format and print the report:

Step	Action
1	• Choose ***File:Report***. or • Click the ***Report*** icon. *Result*: The ***Generate Report*** dialog opens.
2	• Select a ***Format*** for the report.  ***Note:*** *Global report formats are noted by the text "Global" before the report format name in this list.*

Step	Action
3	• Click the ***Preview*** button to view the report in the ***Customize Report*** window and click the ***Print*** icon. or • Click the ***Print*** button. *Result*: The ***Print*** dialog opens.
4	• Select a printer from the ***Printer*** droplist. • Select a ***Print Range***, all pages or just selected pages. • Select a number of copies.
5	• Click the ***OK*** button. *Result*: The report is printed on the selected printer.

Note: *Select **Edit mode** in **Customize Report** to change the layout. You can either print the edited format from this mode and exit **Customize Report** without saving the changes or save the edits when you exit.*

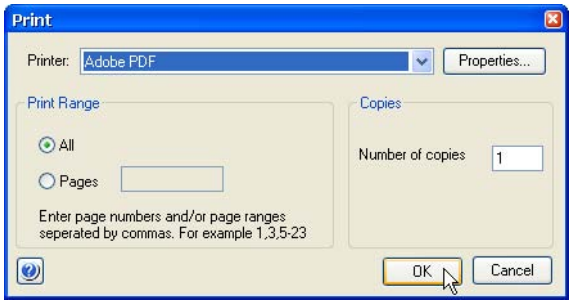
Save the report in PDF format

The generated report can be saved as a PDF file, regardless if Adobe™ Acrobat™ is installed on the computer of not. The two alternatives for saving the report are described below:

Save the report with Adobe Acrobat installed

Step	Action
1	Perform steps 1 to 3 in the *"Generate and print the report"* instruction above.

Step	Action
2	• Select an Adobe PDF printer from the ***Printer*** list.



Note: *You must have a full installation of Adobe Acrobat or a suitable PDF creation application to be able to do this.*

3	• Click the ***Properties*** button and edit the document properties if needed. • Select the print range and number of copies.
4	• Click **OK**. *Result*: The report is created as a PDF file and saved in the location specified in your Acrobat settings.

Save the report without Adobe Acrobat installed

Step	Action
1	Perform steps 1 to 2 in the "*Generate and print the report*" instruction above.
2	Click the ***Preview*** button to view the report in the ***Customize Report*** window
3	Select ***File:Save As PDF***. *Result*: The ***Save As*** dialog opens.
4	• Browse for a folder and ***File name*** for the report. • Click ***Save***. *Result*: The report is created as a PDF file and saved in the location specified in the dialog.

3.6.2 Create a new report format

Introduction

This section describes how to create a new, customized report format. You can choose from a variety of objects to include in a report, including chromatograms, methods, documentation, free text and more. You can also place, align and size the objects as you please.

Note: *Click the **Preview/Edit mode** button to toggle between the **Preview** mode which is for view only, and the **Edit mode** where you can edit the report items. The editing actions in this section are only available in the **Edit mode**.*

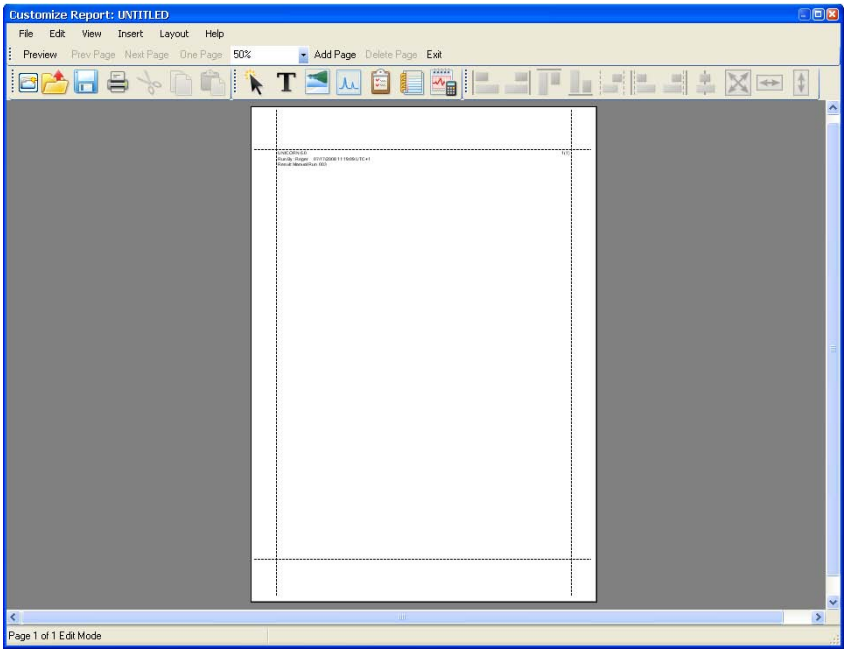
Open the Customize Report window

The table below describes how to open the ***Customize Report*** in ***Edit mode*** to create a new report format.

Step	Action
1	Open a result in the ***Evaluation*** module.
2	• Select ***File:Report***. or • Click the ***Report*** icon. *Result*: The ***Generate Report*** dialog box opens.
3	• Click the ***New*** button. *Result*: The ***Customize Report*** window opens in ***Edit mode***.

The Edit mode window

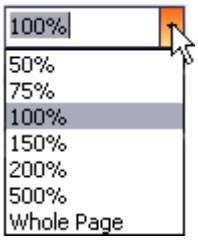
The illustration below shows the ***Customize Report*** window in ***Edit mode*** with a blank report open:


Customize Report: UNTITLED
File Edit View Insert Layout Help
Preview Prev Page Next Page One Page 50% Add Page Delete Page Exit
Page 1 of 1 Edit Mode

Toolbar commands in the Customize Report window

The table below describes the different functions of the toolbar command buttons in the ***Customize Report*** window:

Toolbar button	Function
Preview/Edit mode	This button toggles between a print preview of the report and the ***Edit mode***.
Prev Page	This button displays the previous page or pair of pages (if there is more than one page).
Next Page	This button displays the next page or pair of pages (if there is more than one page).

Toolbar button	Function
One Page/Two Pages	This button toggles between single page view and pairs of pages view (if there is more than one page).
	Select the magnification of the view in this droplist.
Add Page	This button adds a blank page to the report.
Delete Page	This button deletes the current page from the report.
Exit	This button closes the ***Customize Report*** window.

Note: *The general toolbar icons are described below. The toolbar icons for specific formatting operations are described in the instructions for how to use the functions.*

General toolbar icons

The table below describes the different functions of the general toolbar icons in the ***Customize Report*** window:

Toolbar icon	Function
	Opens a new, blank report. ***Note:*** *You can also choose the **File:New** menu command.*
	Opens the ***Open Report Format*** dialog. You can choose to open a previously defined format for editing. ***Note:*** *You can also choose the **File:Open** menu command.*

Toolbar icon	Function
	Saves the edited report format. ***Note:*** *You can also choose the **File:Save** menu command.*
	Cuts the selected object from the report. ***Note:*** *You can also choose the **Edit:Cut** menu command.*
	Copies the selected object in the report. ***Note:*** *You can also choose the **Edit:Copy** menu command.*
	Pastes a copied or cut object from the clipboard into the report. ***Note:*** *You can also choose the **Edit:Paste** menu command.*

Add or delete report pages

The table below describes how to add or delete report pages:

If you want...	then...
to add new pages,	• click the ***Add Page*** toolbar button. *Result*: A new page is added after the last page.
to delete a page while in ***One Page*** mode,	• select the page with ***Next Page*** or ***Prev Page***, • click the ***Delete Page*** toolbar button and confirm the deletion.
to delete a page in ***Two Page*** mode,	• select the page with ***Next Page*** or ***Prev Page***, • click an object on the page, • click the ***Delete Page*** toolbar button and confirm the deletion.

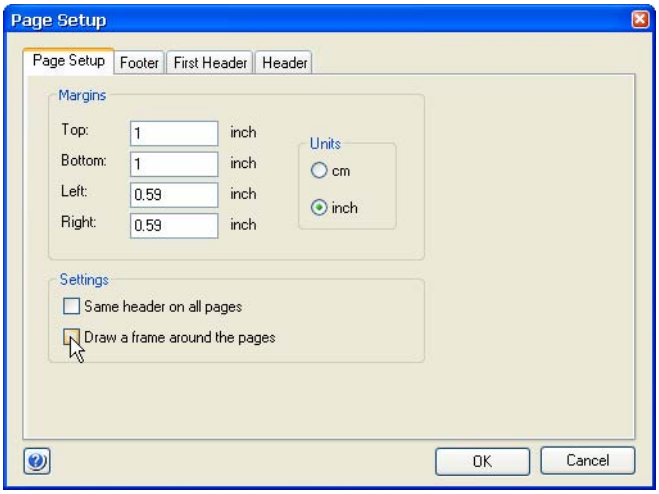
Note:

- *The menu command **Edit:Delete** or the **Delete** key can only be used to delete objects, not whole pages.*
- *The number of pages in the **Edit mode** does not always correspond to the number of printed pages. Click **Preview** in the toolbar for a print preview.*

Change the page layout

The page layout is changed in the ***Page Setup*** dialog. The table below describes how to set up the page layout:

Step	Action
1	• Double-click anywhere on the report page (not on an object) or • Right-click (not on an object) and choose ***Properties*** from the shortcut menu or • Choose the ***Edit:Page setup*** menu command. *Result*: The ***Page Setup*** dialog box opens.



Step	Action
2	• Type new values for the ***Margins*** if necessary. • Select the appropriate ***Settings*** and ***Unit***. ***Note:*** *An extra **Header** tab will appear if you de-select the option to have the same header on all pages. The **First Header** tab is used for the first page header only, and the **Header** tab is used for all subsequent pages.*
3	• Click the ***First Header*** tab. • Select all the items you want to include in the header from the ***Select Items*** list. • Click the ***Font*** button to change the font for all items if necessary.
4	• Type header text in the ***Free text*** box and click the ***Font*** button to alter the default font if necessary. This text will be placed on top of the header. • Type the report title in the ***Report title*** box and click the ***Font*** button to alter the default font if necessary. The title is centered immediately above the page contents.
5	If you want to have a line under or over the header, select the appropriate option in the ***Layout*** field.
6	• Repeat steps 3 to 5 on the ***Footer*** tab and the subsequent pages ***Header*** tab. ***Note:*** *All **Header** and **Footer** tabs contain the same options. You can have all information in either the header or footer or split information between the header and footer as required.*
7	Click ***OK*** to apply the changes.

Add objects to the report

The table below describes how to add objects to the report. The various objects are described below this table.

Step	Action
1	• Click the appropriate icon in the ***Report items*** toolbar. or • Choose an object from the ***Insert*** menu.
2	• Press and hold the left mouse button on the report page, and drag out a box to the size of the item you want to insert. ***Note:*** *The mouse pointer shows a symbol for the type of item you have selected.* • Release the mouse button. *Result*: A ***Setup*** dialog opens. The dialog is specific to the type of item that you want to insert.
3	• Select the desired ***Settings***, for example ***Start on new page***, and click ***OK***. *Result*: The object is inserted onto the page.

Note:

- *If you want to edit an object later, double-click the object box.*
- *The size of the object in **Edit mode** does not always correspond to the size in the printed report. Click **Preview** in the toolbar for a print preview.*

Add free text

The table below describes how to add free text to the report:

Step	Action
1	• Click the ***Free Text*** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the text. Release the button. *Result*: The ***Setup Free Text*** dialog box opens.
2	Type text in the edit field.
3	Select the appropriate ***Settings***: • ***Start on new Page***: the text is to start on a new page • ***Size to content***: the text box should be automatically sized • ***This position on all pages***: the text should appear in the same position on all pages, for example as header and footer text.
4	• Click the ***Font*** button to change the default font. *Result*: The ***Font*** dialog box opens. • Make the necessary changes and click ***OK*** to return.
5	• Click ***OK*** *Result*: The text object is inserted onto the page.

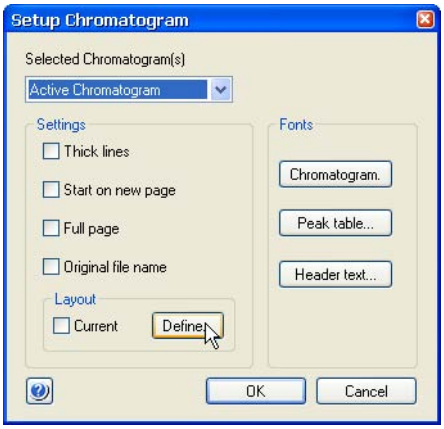
Add a picture

The ***Picture*** dialog box is useful to insert logos, pictures or other figures in the report. The table below describes how to add a picture object to the report:

Step	Action
1	• Click the ***Picture*** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the picture item. Release the mouse button. *Result*: The ***Setup Picture*** dialog opens.
2	• Click the ***Browse*** button to locate the desired picture file. • Select the picture file and click the ***Open*** button. ***Note:*** *A number of image file formats are supported, for example* ***.bmp***, ***.gif***, ***.emf***, ***.jpg*** *and* ***.tif****.* *Result*: A preview of the selected picture is displayed.
3	• Select the desired ***Settings*** (***Start on new Page***, ***Size to content***). • Click ***OK***. *Result*: The picture is inserted onto the page.

Add a chromatogram or peak table

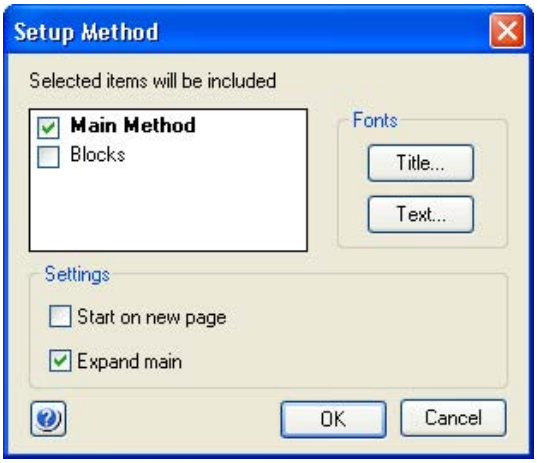
The table below describes how to add a chromatogram and/or peak table to the report.

Step	Action
1	• Click the ***Chromatogram*** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the chromatogram/and or peak table. Release the mouse button. *Result*: The ***Setup Chromatogram*** dialog opens. 
2	Select which chromatogram(s) to insert from the ***Selected chromatogram(s)*** droplist. • ***Active chromatogram*** inserts the chromatogram that currently is active in the ***Evaluation*** module. • ***All chromatograms*** inserts all chromatograms that are open in the ***Evaluation*** module. • ***1***, ***2***...etc., inserts the corresponding chromatogram.

Step	Action
3	• Select the desired ***Settings***. • If desired, change the ***Fonts***. ***Note:*** *Separate fonts can be selected for the **Chromatogram**, the **Peak table** and the **Header text**.*
4	• Click the ***Define*** button in the ***Layout*** field if you want to re-define the layout of the chromatogram. *Result*: The ***Report Customize*** dialog box opens. • Make the appropriate changes and click ***OK*** to return to the ***Setup Chromatogram*** dialog box. ***Note:*** - *All curves can be de-selected in the **Report Customize** dialog box leaving only the selected peak table(s) in the report.* - *The changes that you make will only affect the report and not the view of the chromatograms in the **Evaluation** module.*
5	Click ***OK***. *Result*: The chromatogram is inserted onto the page.

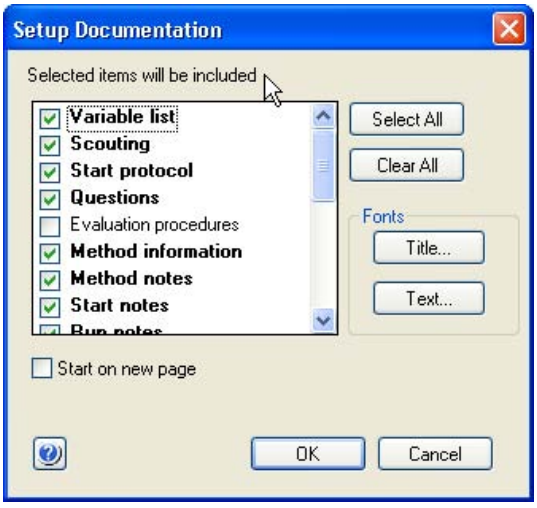
Include a method

The table below describes how to include a method in the report:

Step	Action
1	• Click the ***Method*** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the item. Release the button. *Result*: The ***Setup Method*** dialog opens. 
2	Select the items to be included in the report: • ***Main Method*** is the method on which the run was based. • ***Blocks*** are the blocks that were used in the method.
3	• Select the appropriate ***Settings*** (***Start on new page***, ***Expand main***). ***Note:*** ***Expand main*** *displays the expanded method view.* • If desired, change the ***Fonts*** for the ***Title*** and ***Text***.
4	• Click ***OK***. *Result*: The method object is inserted onto the page.

Add documentation

The table below describes how to add documentation to the report:

Step	Action
1	• Click the ***Documentation*** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the item. Release the button. *Result*: The ***Setup Documentation*** dialog opens. 
2	Select the items to be included in the report: • ***Select All*** includes all items in the report. • ***Clear All*** removes all selections.
3	• If desired, change the ***Fonts*** for ***Title*** or ***Text***. • Select if the documentation should start on a new page.
4	• Click ***OK***. *Result*: The selected documentation items are inserted into the report.

Add the Evaluation Log

The table below describes how to add the ***Evaluation Log*** to the report:

Step	Action
1	• Click the ***Evaluation Log*** icon. • Press and hold the left mouse button on the report page and drag out a box to the size of the item. Release the mouse button. *Result*: The ***Setup Evaluation Log*** dialog opens.
2	• If desired, change the ***Fonts*** for ***Title*** or ***Text***. • Select if the ***Evaluation Log*** should start on a new page.
3	• Click ***OK***. *Result*: The ***Evaluation Log*** is inserted into the report.

Move and resize objects freely

The table below describes how to select, move and resize objects freely:

If you want to...	then...
select a single object,	• click the ***Select*** icon, • click the object of interest.
select several objects,	• click the ***Select*** icon, • press and hold the **Ctrl** key while you click the objects.
move the selected object(s),	click on the objects, hold down the left mouse button and drag the object(s) to the new position.

If you want to...	then...
resize the selected object(s),	click one of the object border anchors, either in the corners or in the middle of a border, and drag the box to the new size. ***Note:*** *Some **Text** objects cannot be resized.*

Alignment toolbar icon functions

Objects can be placed in exact positions and sized in relation to other objects. The table below describes the function of the ***Alignment*** toolbar icons in the **Report Editor**:

Toolbar icon	Function
	Align left Matches the left alignment of all selected objects to that of the highlighted object.
	Align right Matches the right alignment of all selected objects to that of the highlighted object.
	Align top Matches the top alignment of all selected objects to that of the highlighted object.
	Align bottom Matches the bottom alignment of all selected objects to that of the highlighted object.
	Adjust to margins Stretches the selected object(s) to the left and right margins.
	Adjust to left margin Adjusts the selected object(s) to the left margin.

Toolbar icon	Function
	Adjust to right margin Adjusts the selected object(s) to the right margin.
	Adjust to center Adjusts the selected object(s) to the center of the page.
	Make same size Adjusts the selected objects to the same size as the highlighted reference object.
	Make same width Adjusts the selected objects to the same width as the highlighted reference object.
	Make same height Adjusts the selected objects to the same height as the highlighted reference object.

Note: *The **Make same size** and **Make same width** functions can only be used to resize the width of chromatograms, free text and picture objects.*

Save the report format

The table below describes how to save the finished report format:

Step	Action
1	• Choose ***File:Save***. or • Click the ***Save*** icon. *Result*: The ***Save Report Format*** dialog box opens.
2	• Type a name for the format. • Select if you want to save the format for global use. • Select if you want to save the format as default. ***Note:*** *The name for the default format will automatically be changed to* ***DEFAULT****.*
3	• Click ***OK*** to save the format.

3.6.3 Edit an existing report format

Introduction

This section describes how to edit an existing report format.

Note: *By clicking the **Preview/Edit mode** button, you can toggle between a print preview of the report and an editing mode. The editing actions are only available in the **Edit mode**.*

Edit a saved report format

The table below describes how to edit a saved report format in the ***Evaluation*** module.

Step	Action
1	Open a result file.
2	• Select ***File:Report*** or • Click the ***Report*** icon. *Result*: The ***Generate Report*** dialog opens.
3	• Select a report format to edit. • Click the ***Edit*** button. *Result*: The ***Customize Report*** window opens.
4	• Make the necessary changes to the report format. ***Note:*** *Once you have made a change, the title bar of the window will add the word "Modified" to the report format name.*
5	• Select ***File:Save*** or • Click the ***Save*** icon. *Result*: The edited format is saved.

Step	Action
6	To return to the ***Evaluation*** module window: • Select ***File:Exit*** or • Click the ***Exit*** button.
7	• Click the ***Close*** button to close the ***Generate Report*** dialog. or • Select another report format to edit.

Note: *See Section 3.6.2 Create a new report format, on page 74 for instructions about how to add or edit report items. You can also select **File:Save As** in the **Customize Report** window, to save the edited format under another name and keep the original report format unchanged.*

4 Evaluate results

Introduction

This chapter describes how to perform a number of editing and evaluation operations on your results, for example:

- peak integrations
- baseline operations (editing and optimization)
- fraction and peak operations (how to pool fractions, etc.)
- comparison of different runs

For information about how to view results, see chapter *Chapter 3 View and present the results, on page 19*. Curve operations and automated evaluation procedures are described in chapter *Chapter 6 Other evaluation operations, on page 206*.

Note: *Analysis and evaluation of* ***Design of Experiment*** *results is described in the UNICORN 6.0 Method Manual.*

In this chapter

This chapter contains these sections:

Section	See page
4.1 Subtract a blank run curve	94
4.2 Peak integration	98
4.3 Baseline operations	128
4.4 Fraction and peak operations	147
4.5 Compare different runs	159
4.6 The Multi Result Peak Compare wizard	179
4.7 Rename folders, results, chromatograms, curves and peak tables	190
4.8 Sign results electronically	191
4.9 Save results	193

4.1 Subtract a blank run curve

Introduction

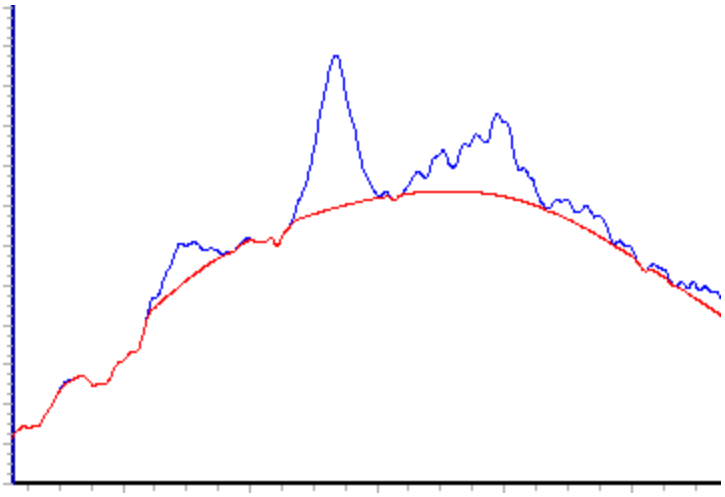
Subtracting a blank run curve is useful to improve the peak integration parameters if the curves have a drifting baseline or ghost peaks.

Ghost peaks

If the ghost peaks come from impurities in the eluents, all equilibration of the columns should be the same from method run to method run. If, for example, the equilibration volume with buffer A is larger before a blank run curve than before a separation, your ghost peaks might be higher in the blank run curve. See *Section 6.1.2 Reduce noise and remove ghost peaks, on page 212* for further information.

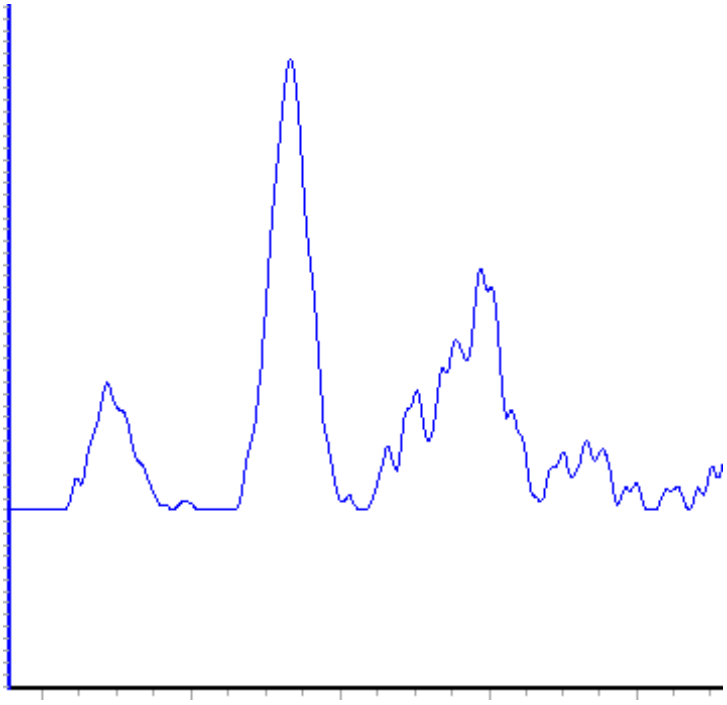
Example of a UV curve with baseline

The illustration below shows a UV curve with a baseline, prior to subtraction of the baseline:



Example of a UV curve after subtraction of the baseline

The illustration below shows the UV curve above, after subtraction of the baseline:



How to import a blank run curve

If a blank run curve was made, this might have been stored in another result file. The table below describes how to import the blank run curve:

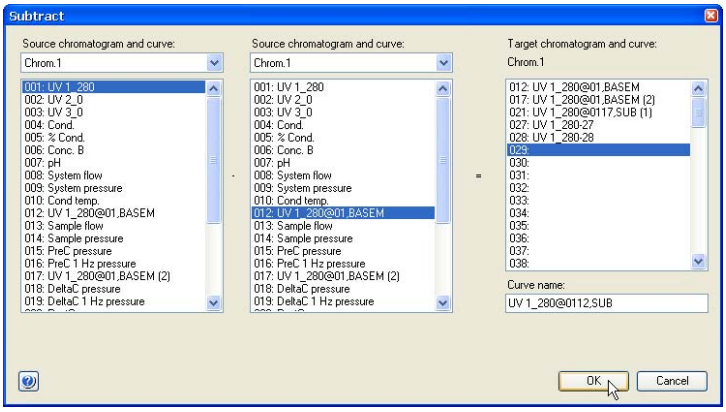
Step	Action
1	Ensure that the destination chromatogram has been opened and is the active tab in the ***Evaluation*** module.
2	Choose ***File:Open:Curves***. *Result:* The ***Open Curves*** dialog box is displayed.
3	Double-click the result file that contains the blank run curve. *Result:* The curves in the first chromatogram are displayed.

Step	Action
4	• Select the appropriate chromatogram in the droplist under ***Available curves***. *Result:* The curves for that chromatogram are displayed in the field below.
5	• Select the curves that correspond to the blank run curve and click the ***Select*** button. *Result:* The selected curve is displayed in the ***Selected curves*** field.
6	• If you want to remove a curve from the field, select it and click the ***Remove*** button. • Click ***OK*** to import the curve.

Note: *If there is no blank run curve available, you can create one with the menu command* ***Integrate:Calculate baseline*** *as described in section* *Section 4.2.2 Perform a peak integration, on page 101**. For more detailed information on how to import curves, chromatograms and other results see* *Section 4.5 Compare different runs, on page 159**.*

Subtract the blank run curve

You can subtract the blank run curve or the baseline from the sample curve. The table below describes how to do this:

Step	Action
1	Select ***Operations:Subtract.*** *Result:* The ***Subtract*** dialog box is displayed. 
2	• Select the sample chromatogram and curve in the chromatogram list and curve field to the left. • Select the baseline or blank run curve to be subtracted in the middle list and field. ***Note:*** *The suggested curve number is highlighted in the* ***Target chromatogram and curve*** *field. A default name is displayed in the* ***Curve name*** *box.* • Click ***OK***.

Note: *All resulting curves from the subtract operation receive the* ***SUB*** *suffix by default. The default curve name can be changed as needed in the* ***Curve name*** *box.*

4.2 Peak integration

Introduction

Peak integration is used to identify and measure a number of curve characteristics including peak areas, retention time and peak widths. This section describes:

- how to calculate a baseline, perform the peak integration and display the data and
- how to perform some special operations, for example peak exclusion.

In this section

This section contains these topics:

Section	See page
4.2.1 Baseline calculation	99
4.2.2 Perform a peak integration	101
4.2.3 Display peak data	104
4.2.4 Edit the integration parameters	108
4.2.5 Integrate part of a curve	121
4.2.6 Exclude or skim peaks	125

4.2.1 Baseline calculation

Introduction

The first step when you integrate peaks is to calculate a baseline. A correct baseline is crucial for accurate calculation of the peak areas. This section describes the options for how to calculate baselines in the ***Peak Integrate*** dialog.

Baseline options

UNICORN offers several options for how to create an accurate baseline:

- To use the automatic ***Calculate baseline*** function.
- To create a baseline based on a blank curve.
- To use a ***Zero baseline***.
- To reuse an existing baseline.

The Calculate baseline function

The ***Calculate baseline*** instruction provides automatic calculation of the baseline. In most cases the measurement is very accurate. The calculation can be performed using the ***Morphological*** algorithm or the ***Classical*** algorithm.

Baselines based on a blank curve

A blank curve can be used as the baseline for peak integration.

- You can use a blank curve with the same chromatographic conditions as the corresponding sample.

  or

- You can subtract the blank run from the source curve and then perform peak integration on the resulting curve with the ***Calculate baseline*** instruction.

Note: *In addition to blank run curves, it is also possible to select any curve from the current chromatogram as the baseline (e.g., an edited baseline.)*

Zero baseline

To use a **Zero baseline** means that there is no baseline subtraction at all. The baseline will be a straight line at the zero level.

Reuse an existing baseline

Using an existing baseline for the selected curve is the default alternative whenever there is an existing baseline available. The option ***Correlated baseline*** is selected if this is the case.

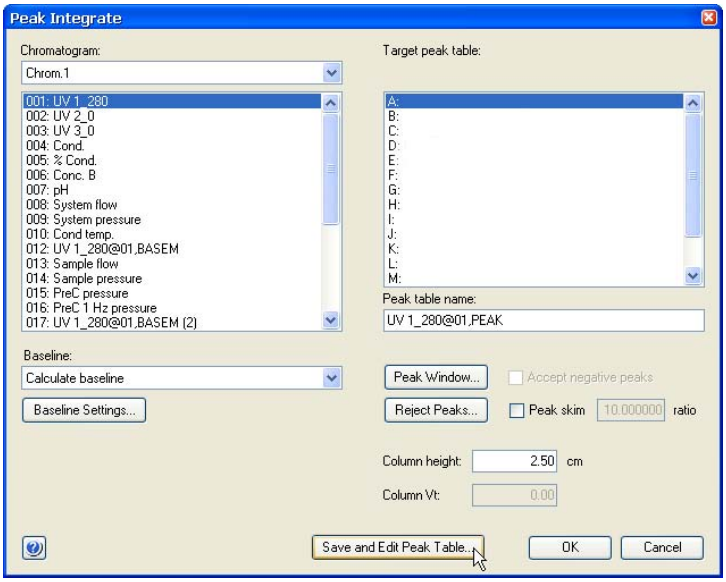
4.2.2 Perform a peak integration

Instruction

The table below describes how to perform a basic peak integration.

Step	Action
1	Open a result file in the ***Evaluation*** module.
2	• Choose ***Integrate:Peak Integrate***. or • Click the ***Peak Integrate*** toolbar icon.

Result: The ***Peak Integrate*** dialog opens.



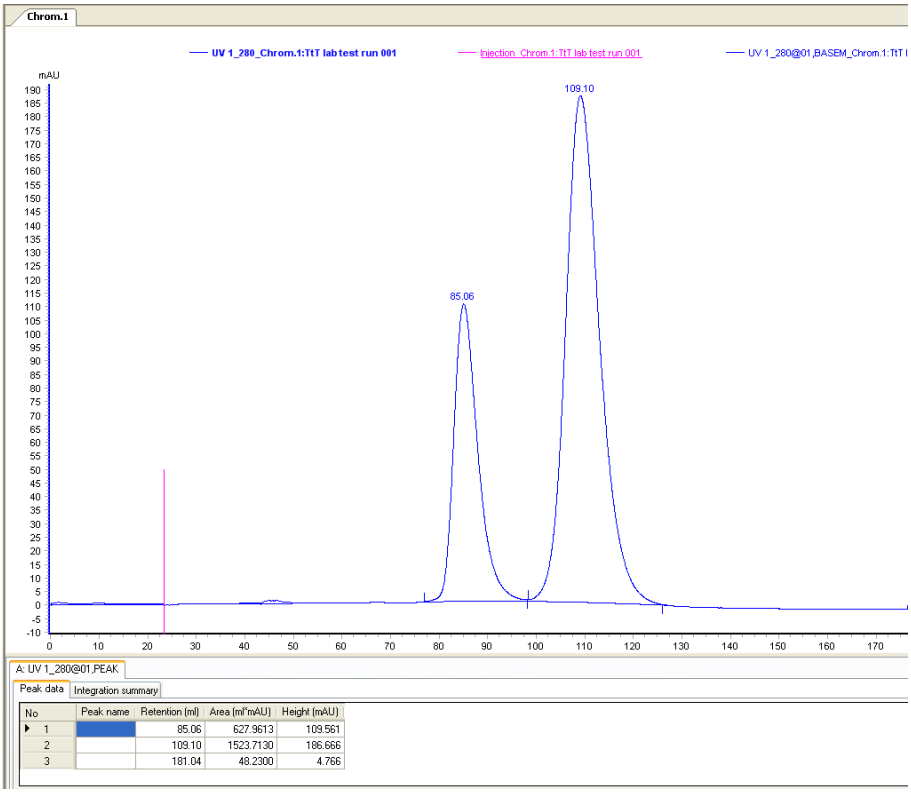
Step	Action
3	• Select a source chromatogram and curve. • Select a baseline or a calculation method from the ***Baseline*** list. • Click ***OK*** to integrate with the default selections. or • Proceed with steps 4 to 6 to change the default selections.
4	• Click the ***Baseline settings*** button to change the calculation algorithm in the ***Settings*** dialog box. The default algorithm is ***Morphological***. • Change the selections or values. ***Note:*** *See also Section 4.3.2 Optimize the baseline with a morphological algorithm, on page 133 and Section 4.3.3 Optimize the baseline with a classic algorithm, on page 138 for more information about how to edit baseline settings.* • Click ***OK***.
5	• Click the ***Peak window*** button to edit the peak window limits if necessary. • Click the ***Reject peaks*** button to set the parameters for peak rejection if necessary (See *Section 4.2.6 Exclude or skim peaks, on page 125*). • Edit the ***Column height*** or ***Column V0*** values if necessary.
6	• Click ***OK*** to integrate and close the dialog box. or • Click ***Save and Edit Peak Table*** to save the integration and open the integrated curve for editing. - See *Section 4.3.1 Edit the baseline manually, on page 129* - See *Section 4.2.4 Edit the integration parameters, on page 108* - See *Section 4.2.5 Integrate part of a curve, on page 121* - See *Section 4.2.6 Exclude or skim peaks, on page 125*

Note: *The peak integrated curve name will end with **PEAK** by default.*

Peak integration results

The peak table is displayed underneath the active chromatogram. The start point and end point of each peak are marked by vertical marks, ***drop-lines***, in the chromatogram. The peaks are automatically labelled according to what is selected in the ***Curve Style and Color*** tab of the ***Chromatogram Layout*** dialog box. A summary of the integration and the settings used can be viewed by clicking the ***Integration summary*** tab.

This is an illustration of the results after a peak integration:


Chrom.1
UV 1_280_Chrom.1:TtT lab test run 001
Injection_Chrom.1:TtT lab test run 001
mAU
109.10
85.06
A: UV 1_280@01,PEAK
Peak data
Integration summary
No | Peak name | Retention (ml) | Area (ml*mAU) | Height (mAU)
1 | | 85.06 | 627.9613 | 109.561
2 | | 109.10 | 1523.7130 | 186.666
3 | | 181.04 | 48.2300 | 4.766

Note: *Peak tables can be copied from one chromatogram to another with the* ***Edit:Copy between chromatograms*** *command. The copied peak table name will have the default ending* ***COPY****.*

4.2.3 Display peak data

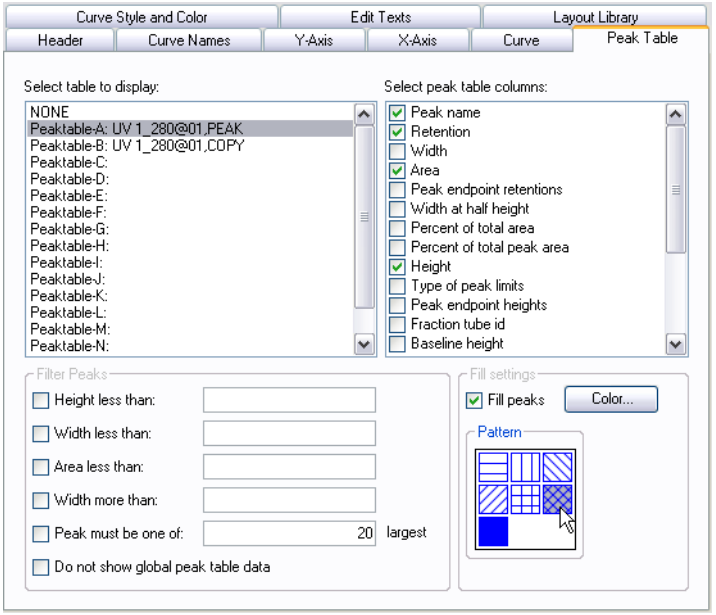
Introduction

There are a number of options for how to display and edit the peak data and peak table contents. This section describes some of these options. Other options are described in *Section 4.2.4 Edit the integration parameters, on page 108*.

Peak characteristics

The peak retention times and several other peak characteristics are calculated automatically. The table below describes how to display other peak characteristics.

Step	Action
1	• Right-click in the active chromatogram. • Select ***Customize*** from the shortcut menu. *Result*: The ***Customize*** dialog opens.
2	Click the ***Peak Table*** tab.



Step	Action
3	• Select options from the ***Select peak table columns*** list. • Click ***OK***. *Result*: The selected items will be displayed in the peak table.

How to filter peaks from view

Peaks can be removed from display in a peak table. The table below describes how to filter the peaks:

Step	Action
1	• Right-click in the active chromatogram or peak table. • Select ***Customize*** from the shortcut menu. *Result*: The ***Customize*** dialog opens.
2	Click the ***Peak Table*** tab.
3	• Click the check boxes in the ***Filter Peaks*** field to select the filter criteria. • Specify filter values. • Click ***OK***.

To filter peaks vs. to reject peaks

The table below describes the major differences in the effects of filtering peaks compared to excluding the peaks by rejection (which is decribed in *Section 4.2.6 Exclude or skim peaks, on page 125*).

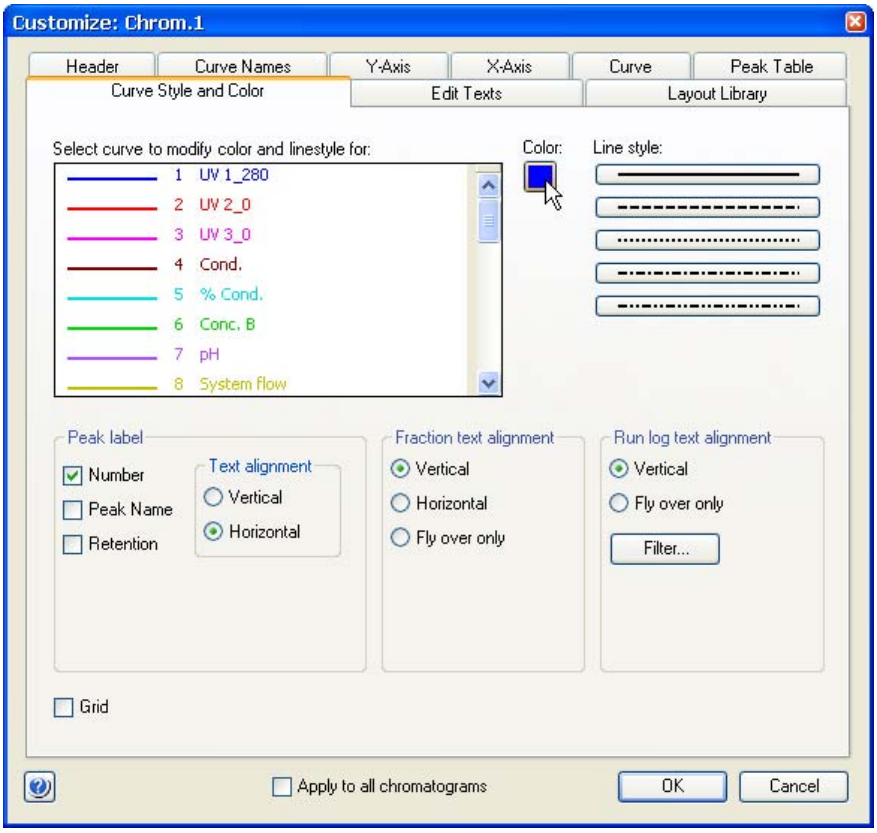
Filter peaks...	Reject peaks...
excludes the peaks from display,	permanently excludes peaks from the integration,
does not exclude the peaks from the calculation of the total peak area,	excludes the peaks from the calculation of the total peak area,
can be reversed.	cannot be reversed.

Peak labels

Peaks can be labelled with their retention, sequentially numbered, or be marked with specific identification names. See the table below for an instruction on how to display peak labels.

The label type can be selected on the ***Curve Style and Color*** tab in the ***Customize*** dialog. De-select all label options to hide the labels, for example for presentations.

The illustration below shows the ***Customize*** dialog with the ***Curve Style and Color*** tab opened:



The table below describes how to display peak labels:

Step	Action
1	• Right-click in the active chromatogram or peak table. • Select ***Customize*** from the shortcut menu. *Result*: The ***Customize*** dialog opens.

Step	Action
2	Click the ***Curve Style and Color*** tab.
3	Select one or more of the following labelling options in the ***Peak label*** field: • ***Number*** *Result*: The peaks will be numbered sequentially. • ***Peak Name*** *Result*: Peak names will be displayed. See *Section 4.2.4 Edit the integration parameters, on page 108* for information about how to name the peaks. • ***Retention*** *Result*: The retention volume or time will be displayed.
4	Select if the label should be aligned vertically or horizontally in the ***Text alignment*** field.
5	Click ***OK***.

Measurement options

It is possible to determine the coordinates of any point on a curve and to obtain values for retention and peak height. This is a useful tool for many other functions, such as for measuring the parameters used in baseline calculations.

Coordinates can be obtained in two ways:

- Through direct measurement using the ***Vertical marker***. This is described in *Section 3.1 Open and view results, on page 20*.
- From peak table data.

4.2.4 Edit the integration parameters

Introduction

Once a peak table has been generated based on an appropriate baseline, it is possible to split or join peaks and to manually adjust the peak start and end points. The peaks will then be renumbered and the peak values will all be recalculated.

The changes are made in the ***Edit Peak Table*** dialog. In this dialog you can also add peak names that may be used as labels in the chromatogram.

Open the peak table for editing

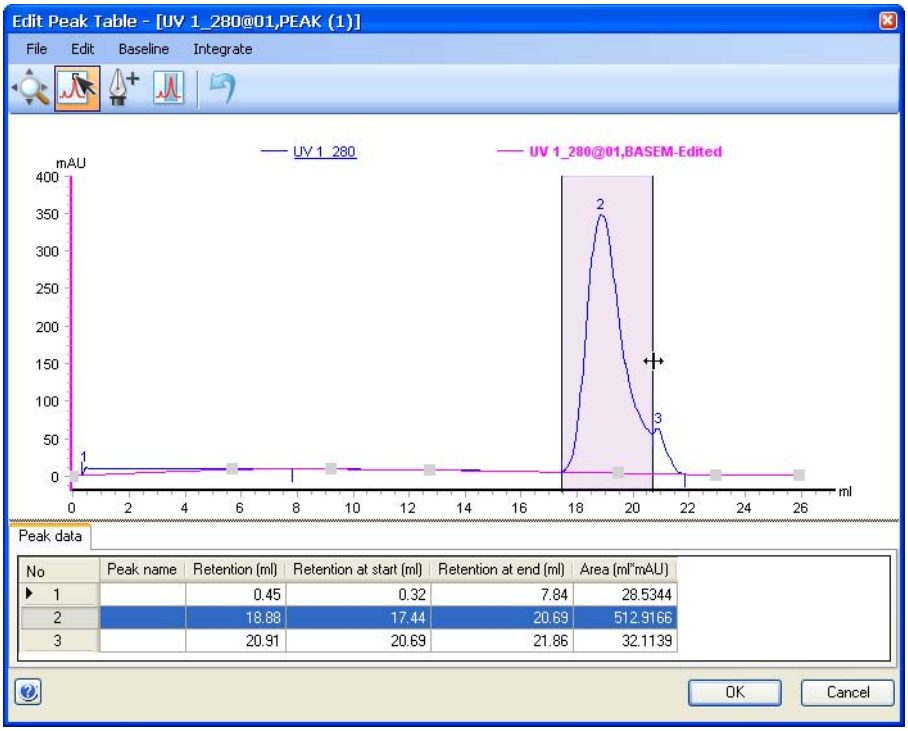
The table below describes how to open the peak table for editing. The editing options are described below.

Step	Action
1	• Select ***Integrate:Edit Peak Table***. *Result*: The ***Edit Peak Table*** dialog opens. ***Note:*** *If there are several peak tables in the result, the dialog will open for the peak table tab that is selected. The name of the baseline on which the peak table was based is displayed in the dialog.*
2	Perform the changes (described in the instructions below).
3	• Click ***OK***. *Result*: The ***Save Edited Peak Table*** dialog opens. The dialog displays a suggested name and location for the peak table.
4	Confirm the name and location and click **OK**.

Note: *The **Edit Peak Table** dialog will be opened immediately if you select **Save and Edit Peak Table** as the last step of the peak integration.*

The Edit Peak Table dialog

The illustration below shows the ***Edit Peak Table*** dialog:



Toolbar icons in the Edit Peak Table dialog

The table below describes the toolbar icons in the ***Edit Peak Table*** dialog.

Icon	Function
	Activates the ***Zoom*** mode. When it is activated, you can use the mouse pointer to drag out an area to be zoomed in. This is described in *The zoom function* below.

Icon	Function
	Activates the ***Edit Peaks*** mode. In this mode, you can select a peak and edit the start and end points. This is described in *Edit the peaks manually* below.
	Activates the ***Set Curve Points*** mode. In this mode, you can select baseline curve points for editing and add new points to re-draw the baseline. This is described in *Adjust the baseline* below.
	Activates the ***Peak Window*** mode. In this mode you can restrict the part of the curve that the peak integration is performed on. See *Section 4.2.5 Integrate part of a curve, on page 121* for more information.
	Returns the result to the state it was in before the last change (***Undo***). ***Note:*** *This function can also be selected in the **Edit** menu.*

The zoom function

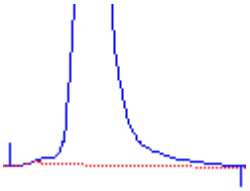
The table below describes how to use the zoom function in the ***Edit Peak Table*** dialog.

Step	Action
1	Click the ***Zoom*** icon. *Result*: The mouse pointer is changed into .
2	• Position the mouse pointer over the left topmost position of the area you want to zoom in on. • Press and hold the left mouse button.
3	Drag the cursor over the area you want to zoom in on.
4	Release the mouse button. *Result*: The area is enlarged.

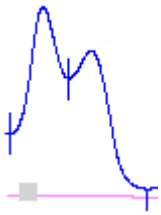
Note: *A **Zoomed mode** note will be displayed in the top righthand corner of the chromatogram when the zoom function has been used. Rightclick and select **Reset Zoom** from the shortcut menu to return to the full display.*

Peak start and end points

The beginning of each peak is marked with a dropline above the curve, and the end of each peak is marked with a dropline below the curve. The illustration below shows an example of start and end point droplines:



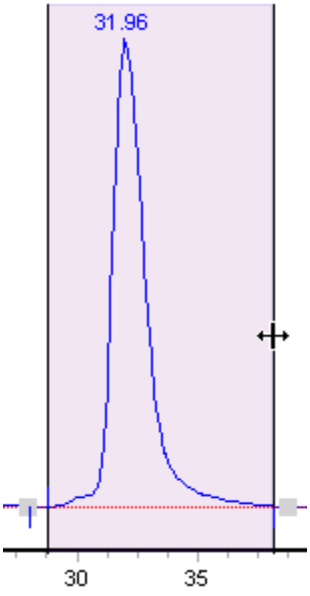
Where there are two adjacent peaks, the end of the first peak will be at the same point as the beginning of the next peak. Thus, there will be a dropline below and above the curve at the same point. See the illustration below:



Edit the peaks manually

The start and end points of a peak can be adjusted graphically by moving the droplines in the ***Edit Peak Table*** dialog. The table below describes this:

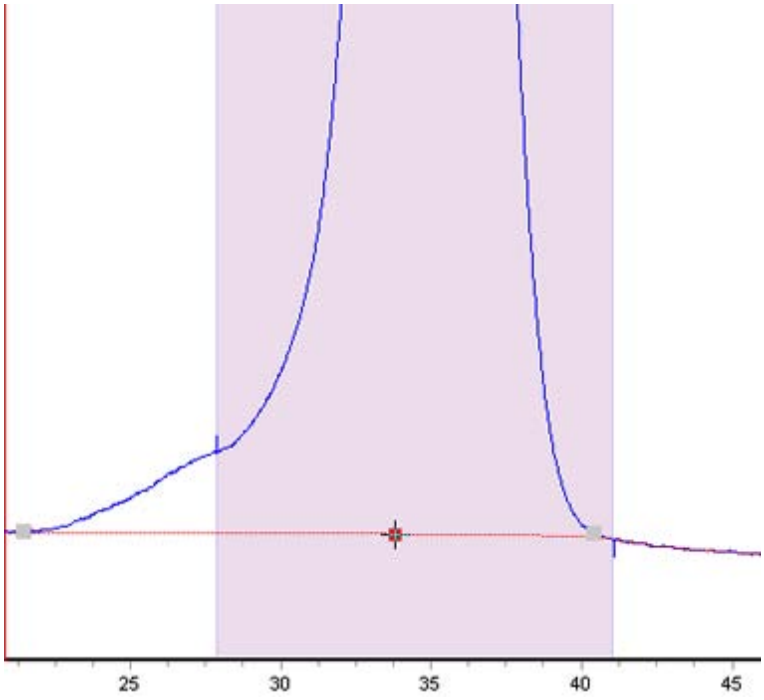
Step	Action
1	Click the ***Edit Peaks*** icon. *Result*: The mouse pointer is changed into ⇩.

Step	Action
2	• Click the peak you want to edit. *Result*: The peak is highlighted and marker lines show the start and end points.
3	• Position the mouse pointer over the marker line that you want to change. *Result*: The pointer is changed into a double-arrow symbol. 
4	• Drag the marker to a new position with the mouse pointer. *Result*: The start or end point droplines of the peak are changed and the corresponding values are updated in the peak table.

Note: *A dropline can never be moved beyond another dropline or beyond a point where the peak meets the baseline.*

Adjust the baseline

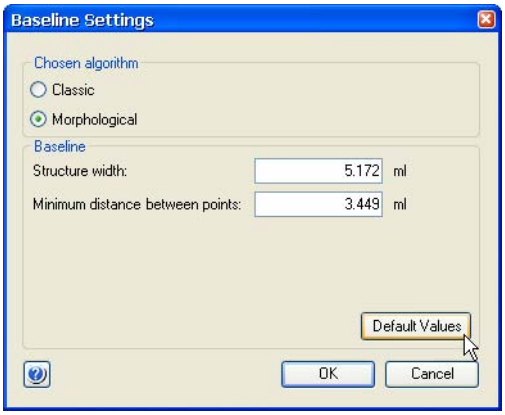
The baseline can be adjusted graphically (see also *Section 4.3.1 Edit the baseline manually, on page 129*) in the ***Edit Peak Table*** dialog. The table below describes this:

Step	Action
1	Click the ***Set Curve Points*** icon. *Result*: The mouse pointer is changed into +.
2	Perform one or more of the operations below as desired: • Click to insert a new data point.  • Doubleclick on a data point or right-click the point and select ***Delete Point*** from the shortcut menu to delete the point. • Click a data point and drag the point to a new position to move the baseline. *Result*: The baseline is edited and the peak table values are recalculated accordingly.

Note: *Accept negative peaks must be selected before the peak integration if you want to be able to drag a data point to move the baseline above the curve.*

Calculate a new baseline

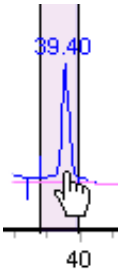
The baseline can be recalculated in the ***Edit Peak Table*** dialog. To alter the baseline settings:

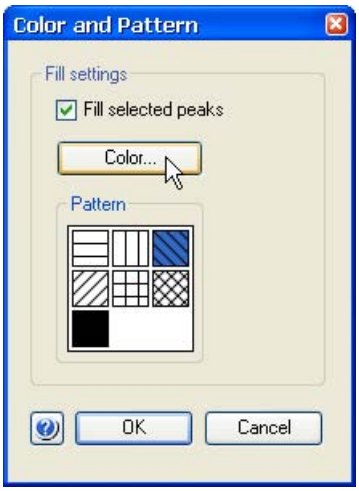
Step	Action
1	• Select ***Baseline:New:Calculate***. *Result*: The ***Settings*** dialog opens. 
2	Select an algorithm (***Morphological*** is default).
3	• Adjust the ***Baseline*** parameters as desired. - Structure width - Minimum distance between points or • Click the ***Default Values*** button for the default values.
4	• Click ***OK***. *Result*: The baseline is recalculated.

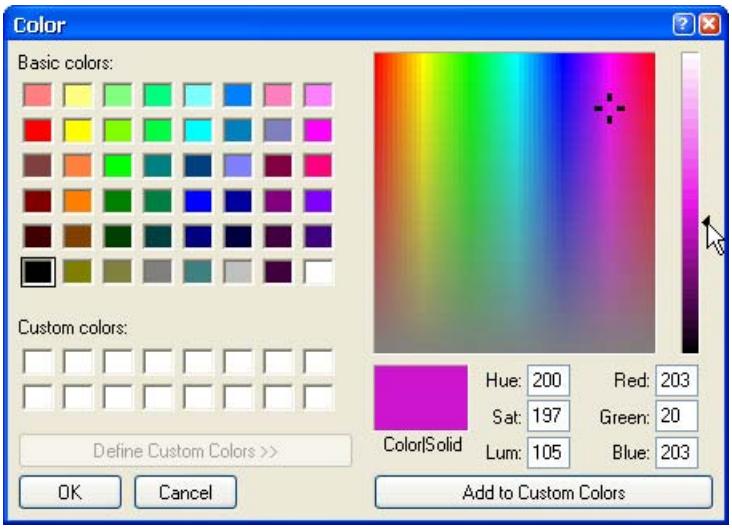
Note: *Select **Baseline:New:Zero Baseline** to replace the calculated baseline with a zero baseline.*

Add color to a peak

The table below describes how to add a fill color and a pattern to an individual peak in the ***Edit Peak Table*** dialog:

Step	Action
1	Click the ***Edit Peaks*** icon. *Result*: The mouse pointer is changed into ⇩.
2	• Move the mouse pointer over the peak you want to edit. *Result*: The mouse pointer is changed into a hand symbol.  • Click to select the peak.

Step	Action
3	• Right-click and select ***Fill Peaks*** from the shortcut menu or • select ***Edit:Fill Peak***. *Result*: The ***Color and Pattern*** dialog opens. 
4	• Select the ***Fill selected peaks*** checkbox. *Result*: The ***Color...*** button and ***Pattern*** field are activated.

Step	Action
5	• Click the ***Color...*** button. *Result*: The ***Color*** dialog opens. 
6	• Select one of the ***Basic colors*** or • click the ***Define Custom Colors >>*** button to open the settings field and define new colors (illustrated above). • After you have selected a color, click ***OK*** to close the dialog.
7	• Select a fill pattern in the ***Pattern*** field. • Click ***OK***.. *Result*: The peak is filled according to the selections.

Note: *The color and pattern selections will override the general **Fill settings** that can be selected for all peaks on the **Peak Table** tab in the **Customize** dialog for the chromatogram.*

Split a peak

It is possible to split a peak into two new peaks by inserting a dropline. The table below describes how to split a peak in the ***Edit Peak Table*** dialog:

Step	Action
1	Click the ***Edit peaks*** icon. *Result*: The mouse pointer is changed into ⇩.
2	Click the peak in the curve or in the peak table to select the peak.
3	• Rightclick and select ***Split Peak*** from the shortcut menu or • select ***Edit:Split Peaks***. *Result*: A new dropline is inserted at the middle point between the two existing droplines and the peak is split. The peak numbering and the peak table are updated accordingly.

Note: *The area under each new peak will not be the same if the symmetry of the original peak was not perfect.*

Join peaks

It is possible to join the areas of adjacent peaks if they are separated by a dropline. The table below describes how to join adjacent peaks in the ***Edit Peak Table*** dialog:

Step	Action
1	Click the ***Edit peaks*** icon. *Result*: The mouse pointer is changed into ⇩.
2	Click the peak in the curve or in the peak table to select the peak.
3	• Right-click and select ***Join Left*** or ***Join Right*** from the shortcut menu or • select ***Edit:Join Left*** or ***Edit:Join Right***. *Result*: The original intervening dropline is removed, all peaks are renumbered and the peak table is updated.

Add peak names

The table below describes how to add names in the ***Edit Peak Table*** dialog, to identify the peaks:

Step	Action
1	Click the ***Edit peaks*** icon. *Result*: The mouse pointer is changed into ⇩.
2	Click the peak in the curve or in the peak table to select the peak.
3	• Right-click and select ***Peak Name*** from the shortcut menu or • choose ***Edit:Peak name*** or • double-click the peak. *Result*: The ***Edit Peak Name*** dialog opens. The number and retention of the selected peak is displayed.
4	Type a name in the ***Peak name*** textbox.
5	• Click ***OK***. *Result*: The new name is added in the corresponding ***Peak name*** cell in the peak table. It is also shown below the peak in the chromatogram. If you have selected to show peak names in the chromatogram, it will also be displayed over the peak.

Note: *You can also add a peak name by selecting the **Peak name** cell in the peak table, click again in the cell and then type the peak name. A pencil symbol is displayed by the peak number to indicate that you are able to enter the text.*

Delete peaks

The table below describes how to delete a peak in the ***Edit Peak Table*** dialog:

Step	Action
1	Click the ***Edit Peaks*** icon. *Result*: The mouse pointer is changed into .
2	Click the peak in the curve or in the peak table to select the peak.
3	• Right-click and select ***Delete Peaks*** from the shortcut menu or • select ***Edit:Delete Peaks*** or • press the **Delete** key. *Result*: The peak is deleted, the remaining peaks are renumbered and the peak table is updated.

The Integrate menu

If needed, you can use the selections on the ***Integrate*** menu to perform a peak integration in the ***Edit Peak Table*** dialog box. This is useful for example if you want to re-integrate the curve using different settings or integrate only part of a curve with different settings.

See *Section 4.2.5 Integrate part of a curve, on page 121* and *Section 4.2.6 Exclude or skim peaks, on page 125* for more information.

4.2.5 Integrate part of a curve

Introduction

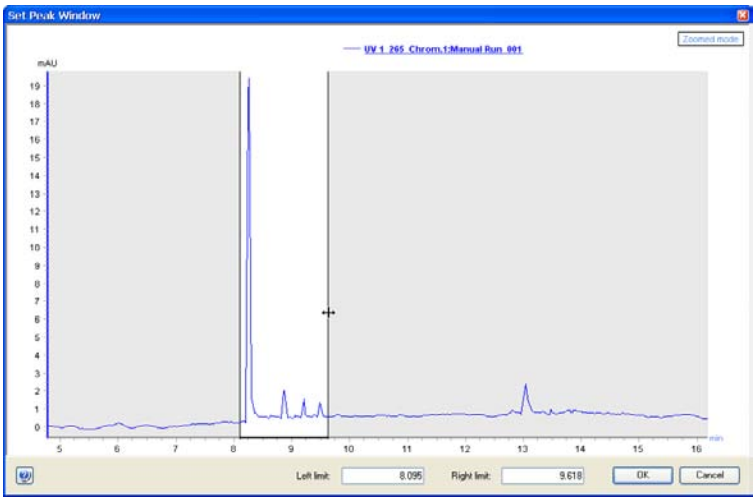
There are several possibilities to improve the results if the peak integration is unsatisfactory. This section describes how to select only part of a curve for integration.

This partial integration can be performed both in the ***Integrate*** dialog in preparation for the peak integration, or in the ***Edit Peak Table*** dialog to adjust an unsatisfactory peak integration. Both alternatives are described here.

Select part of a curve for integration

The table below describes how to select only a part of a curve for peak integration in the ***Integrate*** dialog box:

Step	Action
1	• Choose ***Integrate:Peak Integrate*** or • click the ***Peak Integrate*** icon. *Result*: The ***Peak Integrate*** dialog opens.

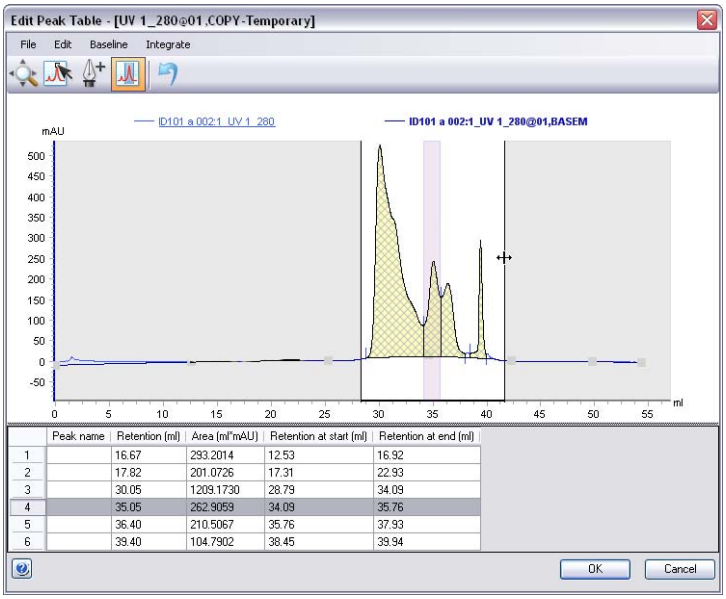
Step	Action
2	• Click the ***Peak Window...*** button. *Result*: The ***Set Peak Window*** dialog opens. 
3	• Type new X-axis values for the ***Left limit*** and the ***Right limit***. or • drag the vertical cursor lines to define the limits.
4	Click **OK**. *Result*: The baseline will be calculated from the whole curve, but the calculation of the peak areas is only performed on the selected section.

Edit integration for part of a curve

Part of a curve can be selected in the ***Edit Peak Table*** dialog box and integrated with settings that differ from the rest of the curve. The table below describes how to do this.

Step	Action
1	• Choose ***Integrate:Edit Peak Table***. *Result*: The ***Edit Peak Table*** dialog box opens.

Step	Action
2	• Click the ***Reintegration Window*** icon. *Result*: The mouse pointer is changed into and two vertical marker lines are displayed. • Place the mouse pointer on one of the marker lines. Result: The mouse pointer changes shape to a double-sided arrow, see picture below.



Note: *The left marker line may be placed over the Y-axis initially.*

3 Drag the cursor lines to the beginning and the end of the selected part of the curve.

Note: *All operations described below will only affect the selected part of the curve.*

Step	Action
4	If desired, change the integration parameters: **Reject peaks** • Choose ***Integrate:Settings***. *Result*: The ***Reject Peaks*** dialog box opens. • Change the settings as desired and click ***OK***. **Skim peaks** • Choose ***Integrate:Peak Skim***. *Result*: The ***Peak Skim*** dialog box opens. • Select the ***Skim Peaks*** checkbox and type a ratio. • Click ***OK***.
5	• Choose ***Integrate:Peak Integrate***. *Result*: The selected part of the curve is peak integrated based on the changed parameters.

Note: *Refer to Section 4.2.6 Exclude or skim peaks, on page 125 for information about the integration parameters in step 4 above.*

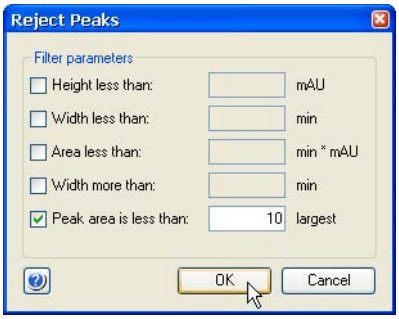
4.2.6 Exclude or skim peaks

Introduction

The results of the peak integration can sometimes be improved if some peaks are excluded. Also, more accurate results may be obtained if smaller peaks are skimmed off larger peaks with shoulders, as described in this section.

Exclude peaks

The table below describes how to define peaks to be excluded in the ***Peak Integrate*** dialog during a peak integration.

Step	Action
1	Click the ***Reject peaks*** button. *Result*: The ***Reject Peaks*** dialog opens. 
2	• Select the appropriate checkboxes and type values for peak height, width and area. • Define how many of the largest peaks you want to include.
3	Click ***OK***.
4	Proceed with the peak integration.

Note: *You can also exclude peaks from the peak integration in the* ***Edit Peak Table*** *dialog. Select* ***Integrate:Settings*** *to open the* ***Reject Peaks*** *dialog.*

Include negative peaks

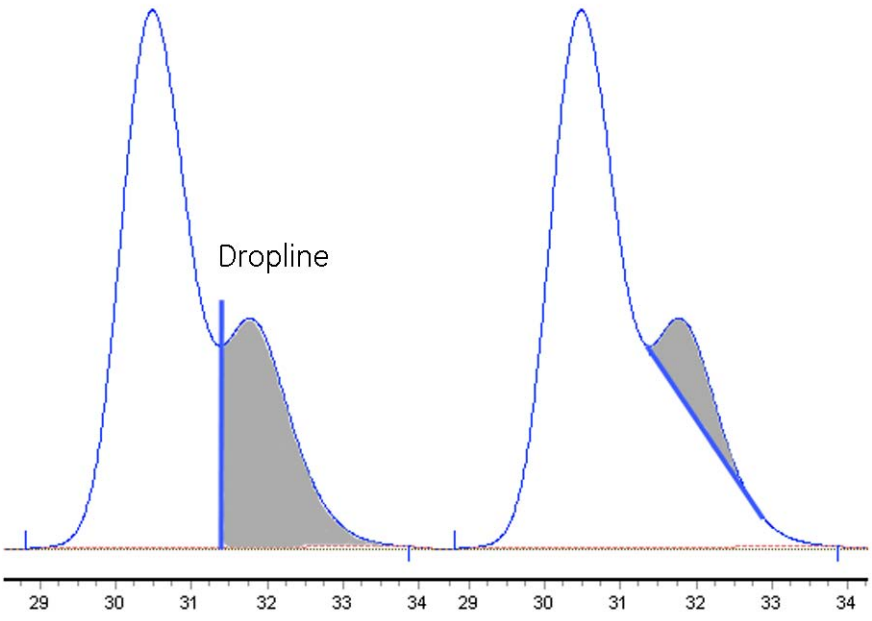
Select the ***Accept negative peaks*** checkbox of the ***Peak Integrate*** dialog to include negative peaks in the integration. The negative peaks will be reported as negative areas in the peak table. By default, negative peaks are not included in the integration.

Peak skimming vs. droplines

The area under a peak can be calculated either using separating droplines or peak skimming:

- ***Droplines*** are vertical marks that split two peaks at the valley. Droplines are used mostly for peaks of relatively similar size. When a peak has a shoulder, splitting with droplines will cause the first peak to lose too much of its area to the peak that forms its shoulder.
- The ***Peak skim*** option can be used to skim off the smaller peak with a straight line that starts in the valley between the peaks and ends at the other side of the smaller peak, at the point where the skim line and the curve slope are equal.

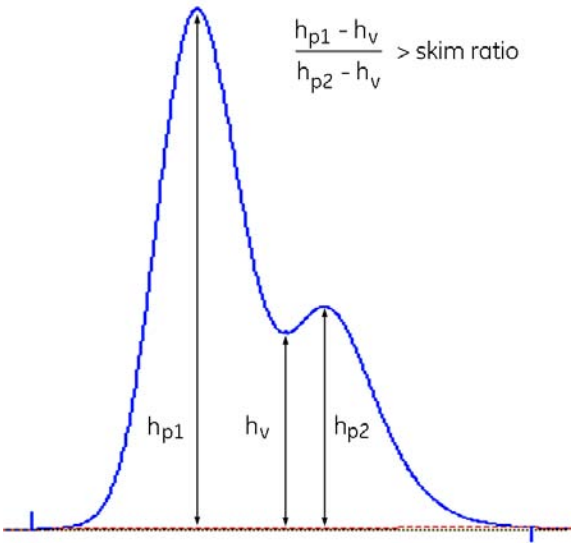
The illustration below is an example of how a dropline (left) and a skimmed peak (right) affect the area under the main peak and the peak shoulder. The peak shoulder area is marked in gray:



Skim peaks

The table below describes how to select a ratio to skim peaks in the ***Peak Integrate*** dialog:

Step	Action
1	Select the ***Peak skim*** checkbox.
2	Determine the ratio when peak skimming should be applied based on the relationship in the illustration below:



Note: *The default ratio value is 10.*

3	Type the ratio value in the text box.
4	Proceed with the peak integration.

Note: *You can set a peak skim ratio or edit earlier settings after the peak integration. Open the **Edit Peak Table** dialog and select **Integrate:Peak Skim...**. and enter a **Ratio** in the **Peak Skim** dialog.*

4.3 Baseline operations

Introduction

In order to achieve the best possible peak integration result, the baseline may have to be edited or optimized. This section describes:

- how to edit the baseline manually

  and

- how to optimize the baseline using a selected algorithm.

In this section

This section contains these topics:

Section	See page
4.3.1 Edit the baseline manually	129
4.3.2 Optimize the baseline with a morphological algorithm	133
4.3.3 Optimize the baseline with a classic algorithm	138

4.3.1 Edit the baseline manually

Introduction

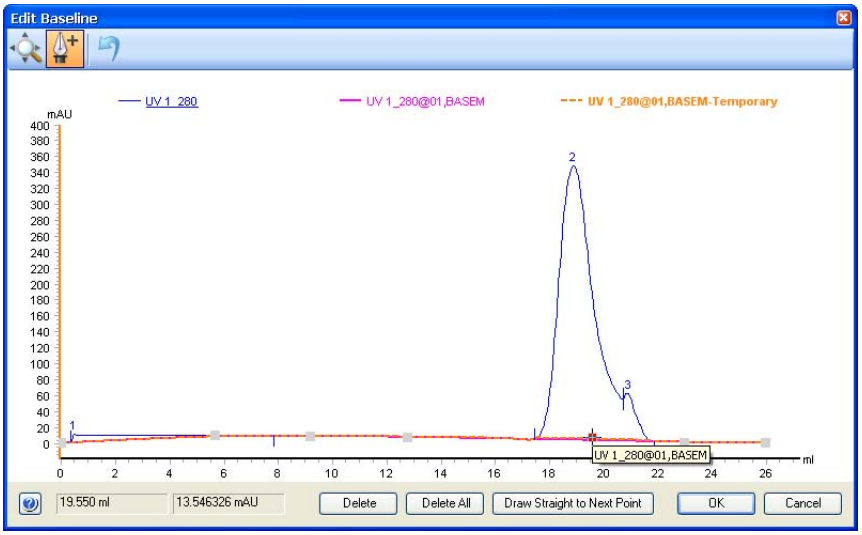
The first choice when you want to optimize the peak integration is to change the baseline parameters. This section describes how to optimize the baseline manually.

The Edit Baseline dialog

You can edit the baseline manually in the ***Edit Baseline*** dialog in the ***Evaluation*** module:

- Select ***Integrate:Edit Baseline*** to display the dialog.

The ***Edit Baseline*** dialog displays the baseline and the curve it was calculated from. The baseline points are marked with gray squares. A selected baseline point is colored red. Hold the cursor above the baseline point to display its coordinates to the left, below the chromatogram. See the illustration below:



How to use the zoom function

The table below describes how to use the zoom function in the ***Edit Baseline*** dialog.

Step	Action
1	• Click the ***Zoom*** icon. *Result*: The cursor is changed into a magnifying glass.
2	• Press and hold the left mouse button. • Drag the cursor over the area you want to zoom in on. • Release the mouse button. *Result*: The area is enlarged. Right-click and select ***Reset zoom*** to restore the full view.

How to edit and insert data points

The tables below describe how to edit and insert baseline data points:

Step	Action
1	Select ***Integrate:Edit Baseline***. *Result*: If there is more than one baseline available, the ***Select Baseline to Edit*** dialog opens. If not, proceed to step 2. • Select the baseline you want to edit from the list. • Click ***OK***. *Result*: The ***Edit Baseline*** dialog opens
2	• Click the ***Set Curve Points*** icon. *Result*: The mouse pointer is changed into a pen symbol and a cross.

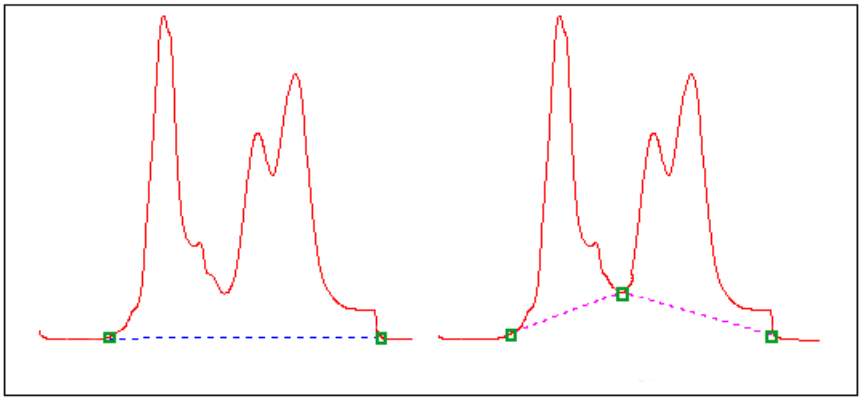
Step	Action
3	Add, delete and/or move data points according to the table below.
6	Click ***OK***. *Result*: The ***Save Edited Baseline*** dialog opens.
7	• Confirm the location and type a new name if necessary. • Click ***OK***. *Result*: The new baseline is saved.

If you want to...	Then...
Add a data point	Click the left mouse button to place a new baseline point in the chromatogram. *Result*: A new point is created, marked by a red square. The baseline curve is redrawn as a smooth spline function based on the old and the new points. The baseline is guided by the points, but does not necessarily pass through them. **TIP:** If you want to create straight baselines, read the instruction later in this section.
Delete a data point	Click the data point to select it and click the ***Delete*** button. *Result*: The data point is deleted and the curve is redrawn. ***Note:*** *Click the **Delete All** button to delete all the data points at once.*
Move a data point	Select the data point and drag it to a new position. *Result*: The baseline curve is redrawn.

TIP: Click the ***Undo*** button to cancel the last edit if the result is not satisfactory.

Edited baseline

The illustration below is a simulated example of a baseline before and after editing:



How to draw a straight line

The table below describes how to force a straight baseline between two points.

Step	Action
1	Select the first of the two points in the point list.
2	Click the ***Draw straight to next point*** button. *Result*: The baseline is drawn through the points as a straight line.

4.3.2 Optimize the baseline with a morphological algorithm

Introduction

The first choice when you want to optimize the peak integration is to change the baseline parameters. This section describes how to optimize the baseline with a morphological algorithm.

The Morphological algorithm

The ***Morphological algorithm*** can be described as a line that follows the chromatogram parallel to the X-axis. Data points for the baseline are created whenever the line touches the curve, and the points are joined at the end to create a baseline.

The ***Morphological algorithm*** gives the best result in curves with drifting baseline and peak clusters. The morphological baseline follows the curve faithfully, and a curve with a baseline at a more even level can be created by subtracting the morphological baseline.

The ***Morphological algorithm*** does *not* work well if there are negative peaks or if quantitative data from negative peaks are important in the run.

Note: *The **Morphological algorithm** is the default baseline setting.*

How to set a Morphological baseline

The table below describes how to choose a ***Morphological algorithm*** and define baseline settings.

Step	Action
1	Select ***Integrate:Peak Integrate***. *Result*: The ***Peak Integrate*** dialog opens.
2	• Choose ***Calculate baseline*** in the ***Baseline*** droplist menu. • Click the ***Baseline settings*** button. *Result*: The ***Baseline Settings*** dialog opens.

Step	Action
3	• Select the ***Morphological*** algorithm. • Change the ***Baseline*** parameters if necessary. See more information about the parameters below this table. • Click ***OK***.

TIP: The same settings can be edited in the ***Calculate Baseline*** dialog when a new baseline is created. Choose ***Integrate:Calculate Baseline*** to open the dialog.

Morphological algorithm parameters

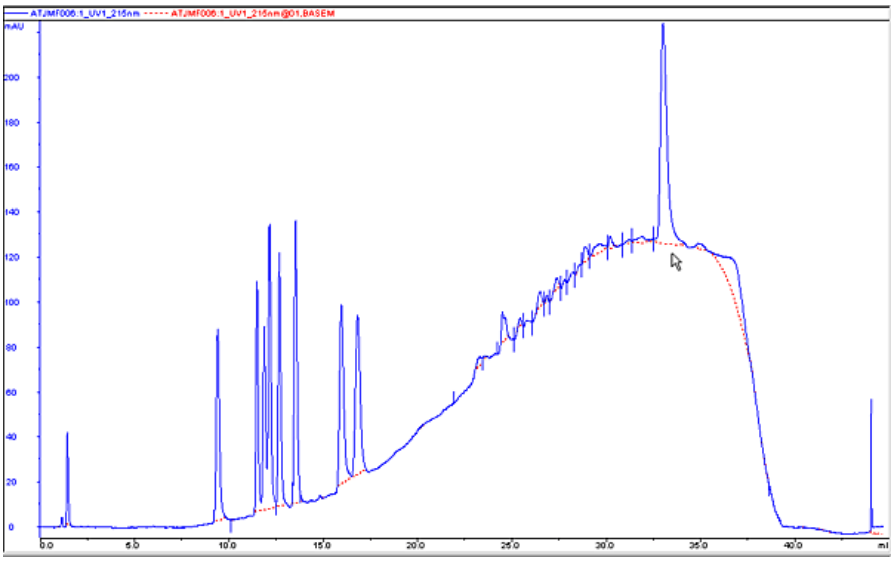
The parameters for the ***Morphological algorithm*** are:

- ***Structure width***
- ***Minimum distance between points***

Structure width

Structure width determines the length of the straight line that follows the chromatogram. The default value is set at the widest peak in the chromatogram multiplied by 1.5.

The illustration below is an example of how a morphological baseline follows the peaks at the different levels in the curve:



The correct structure width settings

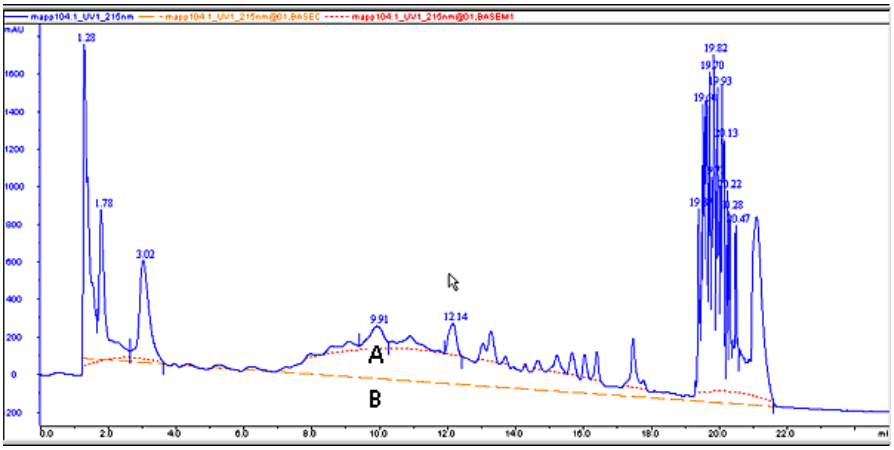
Settings too low

Structure width settings that are too low can result in a baseline that reaches too high up in the peaks of the curve. Sometime a wider peak is not recognized because it contains a cluster of smaller peaks. The ***Structure width*** is then set to a value according to the largest width of the identified narrower peaks, and must be increased.

Settings too high

Structure width settings that are too high mean that narrower peaks, especially in fluctuating curves, are not properly followed. This happens when an artifact in a curve is identified as the widest peak by the morphological algorithm, and then is used to set the default ***Structure width*** value.

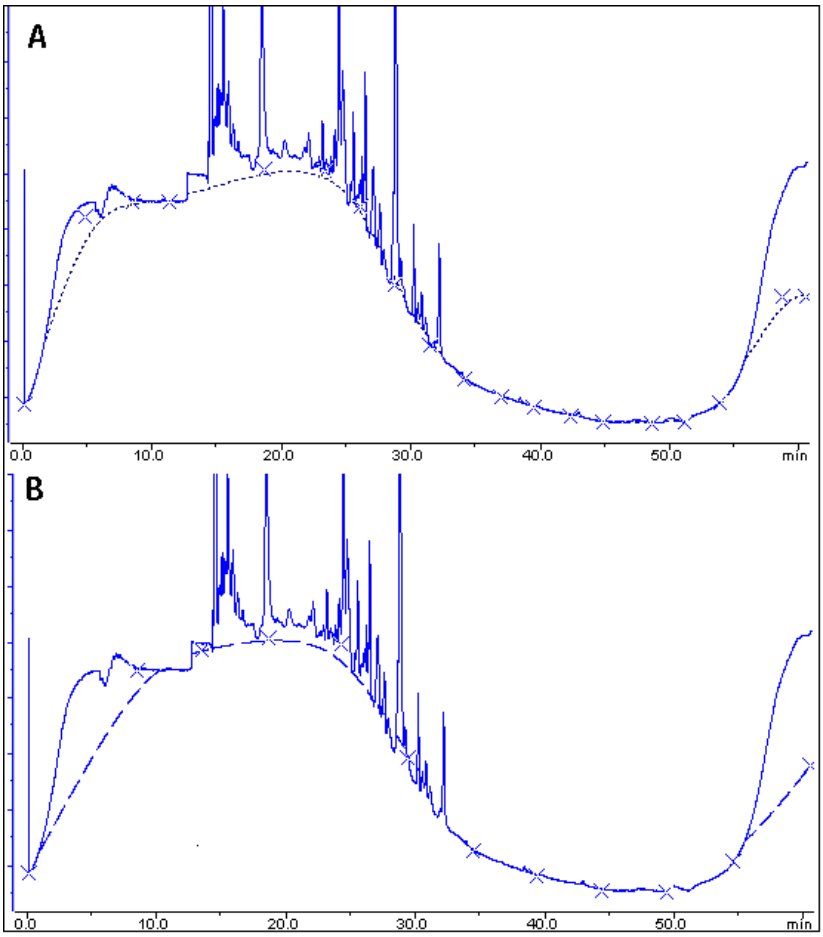
The illustration below is an example of baselines using the default morphological algorithm settings (A) and a morphological algorithm with an increased ***Structure width*** value (B).



Minimum distance between points

The ***Minimum distance between points*** is a measure of the distance between the data points used to generate a baseline. The largest number of data points is produced at the slopes of the curves. If you increase the ***Minimum distance between points*** value, fewer points will be collected on the slopes.

The illustration below is an example of a baseline (A) that is created with the ***Minimum distance between points*** parameter set at a low value. The number of data points is reduced when the ***Minimum distance between points*** parameter is set to a higher value (B).


A
0.0
10.0
20.0
30.0
40.0
50.0
min
B
0.0
10.0
20.0
30.0
40.0
50.0
min

4.3.3 Optimize the baseline with a classic algorithm

Introduction

The first choice when you want to optimize the peak integration is to change the baseline parameters. This section describes how to optimize the baseline with a classical algorithm.

What is the Classic algorithm?

The ***Classic algorithm*** searches for all parts of the source curve that are longer than a defined minimum baseline segment and fall within limiting parameters. Together, the parameter values define the limits for a rectangular box. A part of the source curve must fit entirely inside this rectangular box to be identified as a baseline segment.

The ***Classic algorithm*** is particularly useful when you need to integrate curves with negative peaks and when quantitative data from negative peaks are important.

Classic algorithm parameters

The parameters for the ***Classic algorithm*** are:

- ***Shortest baseline segment***
- ***Noise window***
- ***Max baseline level***
- ***Slope limit***

See more information about the parameters below.

How to set a Classic baseline

The table below describes how to set a ***Classic algorithm*** and define a baseline.

Step	Action
1	Click the ***Baseline settings*** button in the ***Peak Integrate*** dialog. *Result*: The ***Baseline Settings*** dialog opens.

Step	Action
2	• Select the ***Classic*** algorithm. • Change the ***Baseline*** parameters. See more information about the parameters below this table. • Click ***OK***.

TIP: The same settings can be edited in the ***Calculate Baseline*** dialog when a new baseline is created. Choose ***Integrate:Calculate Baseline*** to open the dialog.

Test your parameter changes

The best way to optimize the baseline is to change the baseline parameters step by step and then check the resulting baseline after each change. When the desired effect is accomplished it is best to go back and try a parameter value inbetween the two last settings to avoid an unnecessarily low or high value.

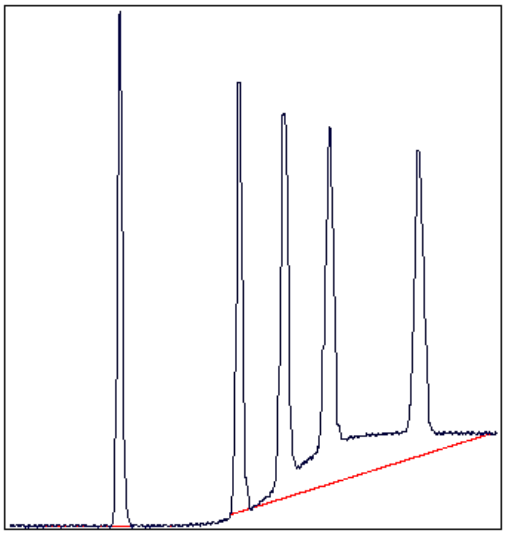
How much the values should be changed depends on the cause of the peak integration problem. The table below is a general guideline.

Baseline parameter	Recommended initial change
Shortest baseline segment	20%-50%
Noise window	10%-30%
Max baseline level	Usually not necessary to adjust
Slope limit	25%-50%

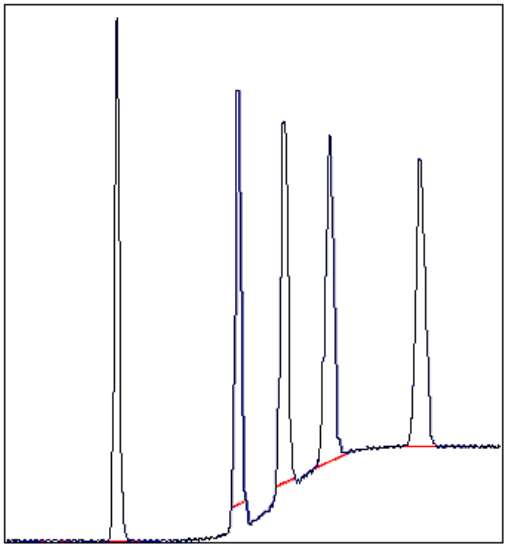
TIP: If necessary, click the ***Default Values*** button to restore the default values.

Shortest baseline segment

If an excessively high ***Shortest baseline segment*** value is set, short curve segments between peaks in the middle of the chromatogram are not identified as baseline segments. The calculated baseline does not follow the source curve, see below:



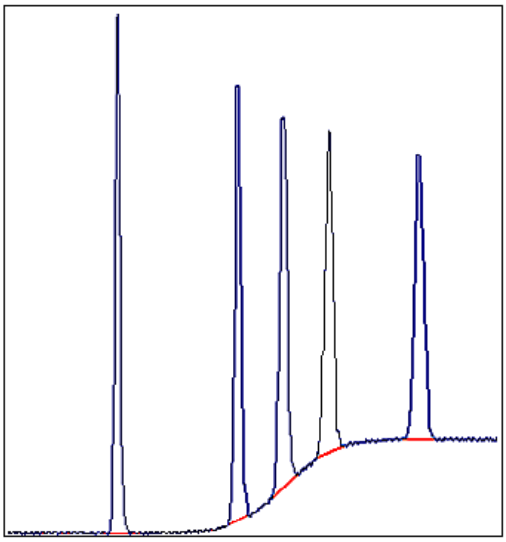
The ***Shortest baseline segment*** value is decreased by 50% in this example:



Slope limit

A changed ***Slope limit*** will often improve the baseline calculation. The ***Slope limit*** sets the maximum slope of the curve to define when a peak is recognized. An excessively high ***Slope limit*** will cause the up-slopes of the peaks to be recognized as baseline segments.

The example above was improved by the shorter baseline segments but the high slope of the short segments in the region between the second and the fourth peak still makes the baseline unacceptable. In the example below the ***Slope limit*** is increased by a factor of 2.5, which produces a correct baseline:

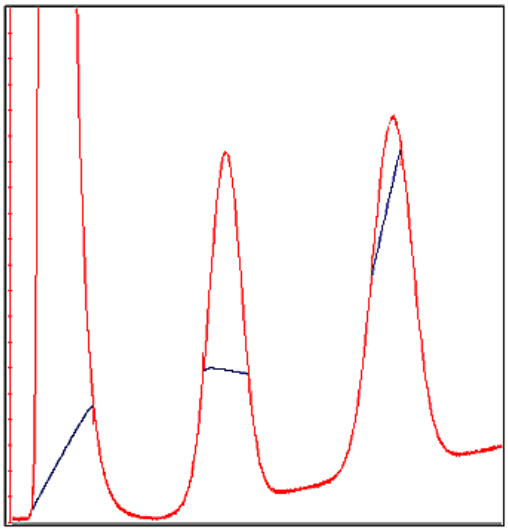


Slope limit too high

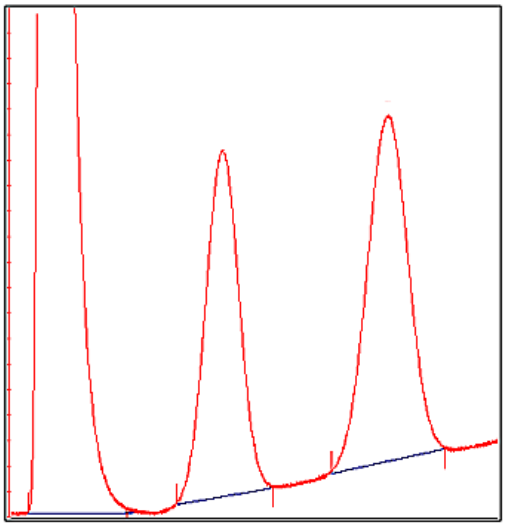
An excessively high ***Slope limit*** value can cause peak limits too high up on the peaks. This can be the case when the chromatogram includes a very large flowthrough or solvent peak. The large peak affects the calculation of the default parameters and leads to excessively high values for the ***Slope limit***.

Note: *An excessive value for the* ***Noise window*** *can have the same effect and be caused by the same situation, often also in combination with a high* ***Slope limit****.*

Peak limits are defined on peaks in the example below due to the high ***Slope limit***:

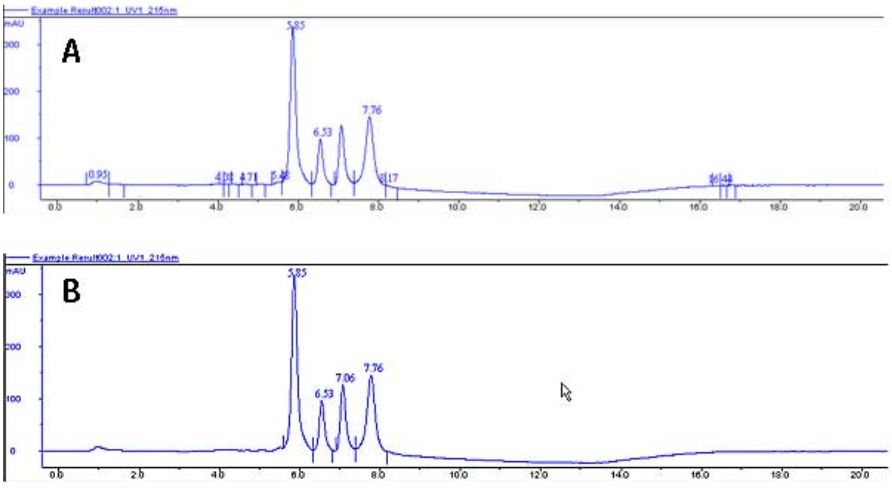


The example below has a much lower ***Slope limit***, and a lower ***Noise window***:



Noise window

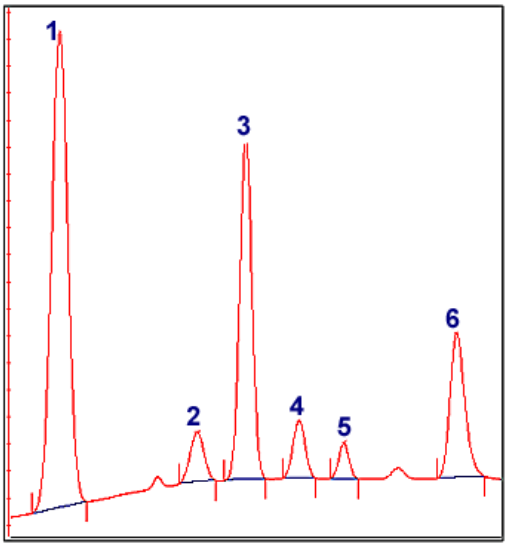
Occasionally too many peaks occur after the peak integration, usually because noise on the baseline is erroneously detected as peaks. The solution to this is to increase the ***Noise window*** parameter. However, this can result in peak limits too high up on the peak slopes. The illustration below is an example of noise detected as peaks (A) and the result of a second peak integration with an increased ***Noise window*** (B).



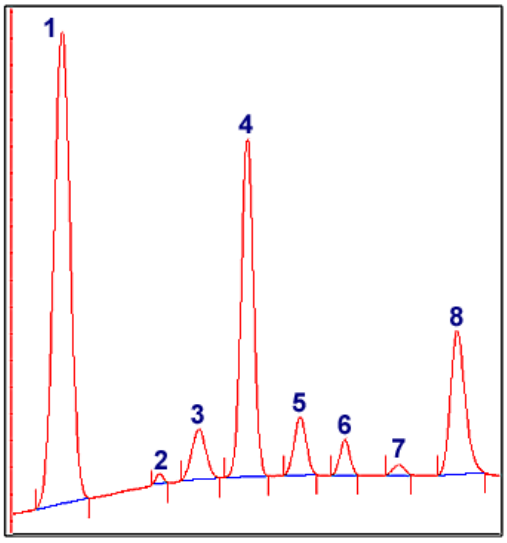
TIP: You can also use the ***Reject peaks*** function in the ***Peak Integrate*** dialog to reduce the number of peaks based on the total number of accepted peaks or the minimum peak height.

Missing peaks

Sometimes obvious peaks are not detected in the peak integration. The probable cause is that the ***Noise window*** is set too high. See the illustration below:



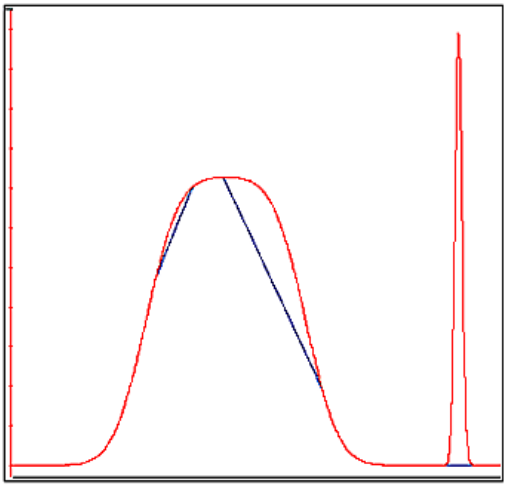
All peaks are detected if the ***Noise window*** is decreased- see example below:



Note: *Missing peaks can also be caused by improper settings for* ***Reject peaks*** *in the* ***Peak Integrate*** *dialog, or* ***Filter peaks*** *in the* ***Chromatogram Layout*** *dialog.*

When to change the Max baseline level

In rare cases the top of a broad, flat peak can be incorporated as a baseline segment. This is one of the very few situations where it is useful to change the ***Max baseline level***. The illustration below is an example:



How to set the Max baseline level

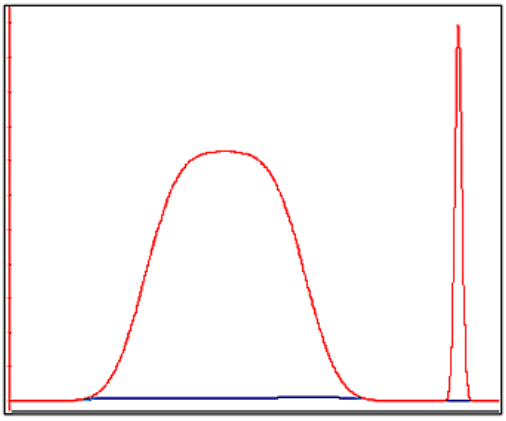
The table below describes how to set the ***Max baseline level***.

Step	Action
1	Right-click in the chromatogram and select ***Marker***. *Result*: A vertical line is set in the chromatogram. A text box in the top left corner of the chromatogram displays the X-axis and Y-axis values of the curve at the point where the vertical ***Marker*** line crosses the curve.
2	• Move the ***Marker*** with your mouse. • Measure the height of the peak you want to exclude from the baseline.

Step	Action
3	Choose ***Integrate:Calculate baseline***.
4	• Select ***Classic*** as ***Algorithm***. • Type a new value for ***Max baseline level***. Set the level slightly lower than the value that you measured in step 2. • Click ***OK***.

Example of a correct baseline

The illustration below is an example of a correct baseline after the ***Max baseline level*** has been changed:



4.4 Fraction and peak operations

Introduction

This section describes other operations that you can perform on your results, including:

- Pool several fractions into combined fractions,
- create an average fraction absorbance curve, the ***Fraction Histogram***,
- match protein activity to a curve using the ***Activity Histogram***
  and
- use the absorbance ratio to verify peak purity or identify peaks.

In this section

This section contains these topics:

Section	See page
4.4.1 Pool fractions	148
4.4.2 The Fraction Histogram	151
4.4.3 Match protein activity to a curve	154
4.4.4 Peak purity and peak identification	156

4.4.1 Pool fractions

Introduction

Fractions are collected sequentially during a separation. Each fraction contains a set volume of sample. This section describes how to pool the curves from several fractions into a new curve.

How to display the fractions in the chromatogram

Each fraction is numbered according to its order in the sequence. The information is saved as a curve under the name ***Fractions.***

To display the position of each fraction in relation to the information displayed on the UV detection curve:

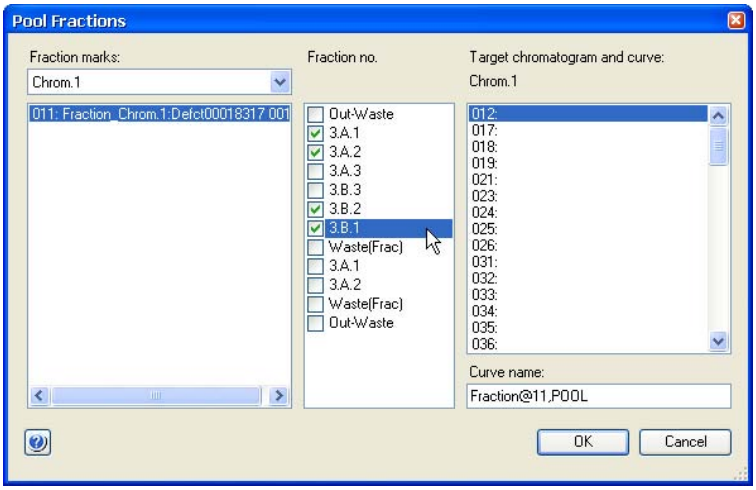
- Select the ***Fraction...*** curve on the ***Curve*** tab in the ***Customize*** dialog.

  or

- Right-click in the chromatogram and select the ***Filter Curves*** menu command. Select the checkbox for the ***Fraction...*** curve.

How to pool fraction curves

The table below describes how to pool fractions.

Step	Action
1	• Open the result from the ***File Navigator***. • Choose ***Operations:Pool Fractions***. *Result*: The ***Pool Fractions*** dialog opens:  The fraction curves for the selected chromatogram are listed in the ***Fraction marks*** field. The fractions for the selected fraction curve are shown in the ***Fraction no.*** field.
2	• Select the check box for each fraction to be pooled. ***Note:*** *Only adjacent fractions will be pooled. In the example above, fraction **3.A.1** will be pooled with fraction **3.A.2** and fraction **3.B.2** will be pooled with fraction **3.B.1**.* • Select the destination for the new curve in the ***Target chromatogram and curve*** field. • Click ***OK***. *Result*: The fraction curve with the pooled fractions is added to the chromatogram.

TIP: The default name for the fraction curve is shown in the ***Curve name*** field. The default file ending is ***POOL***. The default curve name can be changed as needed by typing a new name in the field before the fractions are pooled.

How to show only the pooled fraction curves

The active chromatogram will now show both the original and the pooled fraction curves. The table below describes how to show only the pooled fractions.

Step	Action
1	• Right-click in the chromatogram and select the ***Customize*** dialog. Click the ***Curve*** tab. or • Right-click in the chromatogram and select the ***Filter Curves*** menu command. Select the checkbox for the ***Fraction...*** curve.
2	• De-select the check box for the original fraction curve (remove the check mark). *Result*: The original fraction curve is de-selected and is not displayed.

4.4.2 The Fraction Histogram

Introduction

The ***Fraction Histogram*** dialog in the ***Evaluation*** module can be used to create a curve for the average fraction absorbance. It can also be used to create a table showing the amount of protein and the concentration in each fraction.

Create a Fraction Histogram curve

The table below describes how to create a ***Fraction Histogram*** curve.

Step	Action
1	Select ***Operations:Fraction Histogram***. *Result*: The ***Fraction Histogram*** dialog opens.
2	• Select the desired UV curve in the left ***Source chromatogram and curve*** field. **TIP:** The fractions curve should already be selected on the middle field. If you have previously pooled fractions and created a pooled fraction curve, select the desired fraction curve.
3	• Click ***OK***. *Result*: The average fraction absorbance values are displayed as a new curve in the chromatogram.

TIP: By default, the ***Fraction Histogram*** curve will be assigned to the first available curve position, which is shown in the ***Target chromatogram and curve*** list. A default curve name with the ending ***HIST*** is suggested in the ***Curve name*** field. You can choose another curve position and type another curve name in the ***Curve name*** field if desired.

Protein concentrations in the fractions

The protein concentration in the fractions are calculated using the following formula:

*Concentration [mg/ml] = A / (d * 1000 * Ext. coef.)*

A = *Average fraction absorbance* = ***Area / Volume [mAU]***

d = *UV cell path length [cm]*

Ext. coef. = Protein coefficient at used wavelength. [l g^{-1} cm^{-1}]

Protein amounts in the fractions

The total amount of protein found in the fraction is calculated using the following formula:

*Amount [mg] = Concentration [mg/ml] * fraction volume [ml]*

Calculate the protein concentration and amount

The table below describes how to calculate the protein concentration and amount in the fractions using the ***Fraction*** table in the ***Fraction Histogram*** dialog:

Step	Action
1	Select ***Operations:Fraction Histogram***. *Result*: The ***Fraction Histogram*** dialog opens. The UV path length is automatically shown in the ***Path length*** field.
2	Type the extinction coefficient for each fraction in the corresponding ***Ext. coef*** table cell. *Result*: The fraction protein concentration and amount are calculated and displayed in the corresponding ***Conc.*** and ***Amount*** table cells. ***Note:*** *The values are calculated using a zero baseline (i.e., no baseline subtraction is applied).*

Export the Fraction table

The complete ***Fraction*** table can be exported as an Excel file:

Step	Action
1	Click the ***Export Table*** button in the ***Fraction Histogram*** dialog. Result: The ***Export Fraction Table to Excel*** file dialog opens.
2	Browse to the folder where you want to save the file.
3	Type a name in the ***File name*** field.

Step	Action
4	Click the ***Save*** button. *Result:* The table is saved in Excel format.

Print the Fraction table

The complete ***Fraction*** table can be printed directly from the ***Fraction Histogram*** dialog:

Step	Action
1	Click the ***Print Table*** button. *Result:* The ***Print*** dialog opens.
2	If necessary, select a printer and the number of copies to print. You may also click the ***Properties*** button in this dialog, to change the general printer settings.
3	Click ***OK***. *Result:* The table is printed on the selected printer.

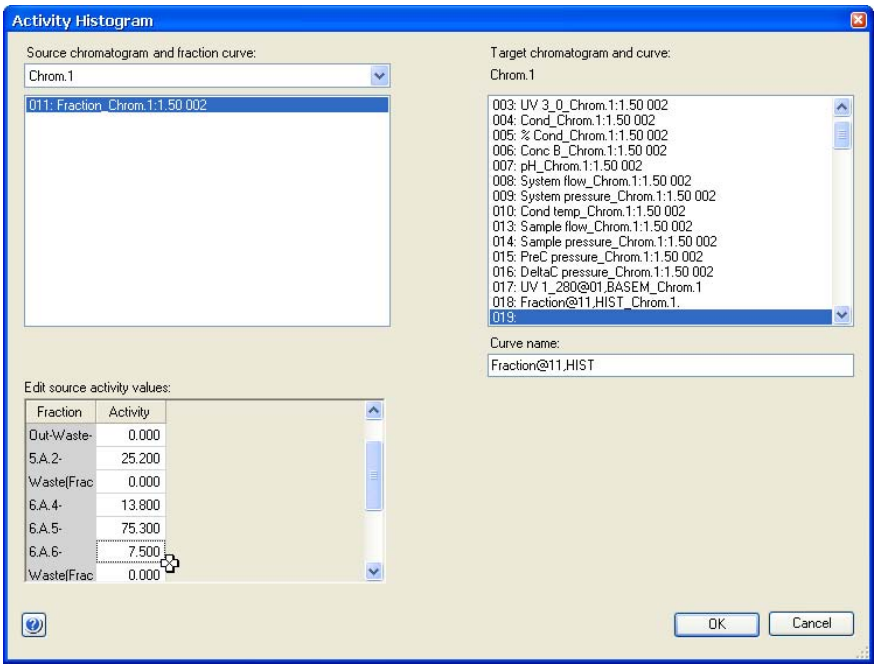
4.4.3 Match protein activity to a curve

Introduction

You can compare data from the results of protein activity assays, such as ELISA, with the data contained in the UV curve. The activity curve and the UV curve can be compared in a combined presentation.

The Activity Histogram dialog

The illustration below shows the ***Activity Histogram*** dialog:



Enter protein activity values for comparison

The table below describes how to enter the values from a protein activity assay in a comparison histogram:

Step	Action
1	Choose ***Operations:Activity Histogram***. *Result*: The ***Activity Histogram*** dialog opens.
2	By default, the fraction curve for the current chromatogram is selected in the ***Source chromatogram and fraction curve*** list. • If necessary, change the source and target chromatograms. All the component fractions of the fraction curve are listed in the ***Fraction*** column of the ***Edit source activity values*** field.
3	• Type an activity value for each fraction in the ***Activity*** column. • Click ***OK***. *Result:* A histogram curve showing the activity values is added to the chromatogram.

TIP: By default, the ***Activity Histogram*** curve will be assigned to the first available curve position, which is shown in the ***Target chromatogram and curve*** list. A default curve name with the ending ***HIST*** is suggested in the ***Curve name*** field. You can choose another curve position and type another curve name in the ***Curve name*** field if you so wish.

4.4.4 Peak purity and peak identification

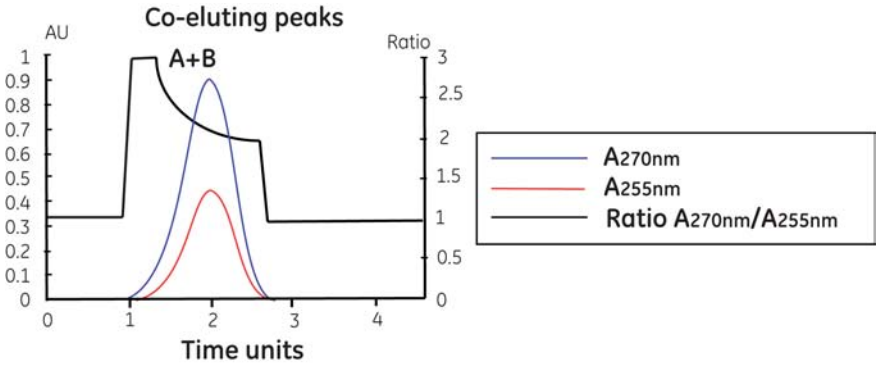
Introduction

Ratios between UV curves measured at different wavelengths give useful information about peak purity or peak identity.

Peak purity

The absorbance ratio can be used to check peak purity. If the peak is pure, the absorbance spectra are the same over the whole peak and the ratios should therefore remain constant. The peak is probably not pure if the absorbance ratio is not the same over the whole peak.

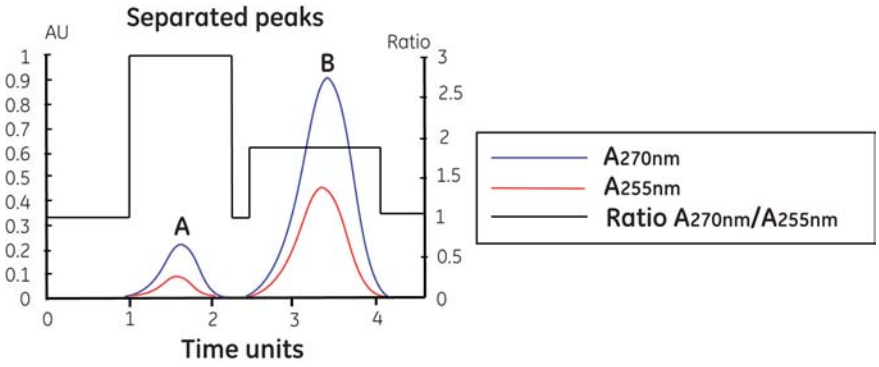
The illustration below shows a chromatogram of two co-eluting components with differing absorbance spectra and a small difference in retention time:



Peak identification

The absorbance ratio can be used for peak identification. Different compounds have a specific ratio between absorbancies at different wavelengths.

The illustration below shows a chromatogram of two components with differences in their absorbance spectra:



Divide the curves

Both curves must have a baseline close to zero AU before they can be divided. This is achieved with baseline subtraction. The table below describes how to subtract the baseline from an earlier integration and divide the curves:

Step	Action
1	Perform peak integrations to create a baseline for each UV curve, see Perform a peak integration.
2	Select ***Operations:Subtract***. *Result*: The ***Subtract*** dialog opens.
3	• Select the UV curve in the first ***Source chromatogram and curve*** list. • Select its baseline in the second ***Source chromatogram and curve*** list. • Click ***OK***. *Result:* A curve with the subtracted baseline is added to the chromatogram in the first available curve position. By default, the curve name will have a ***SUB*** ending. **TIP:** You can also subtract corresponding blank runs if there are blank runs available.
4	Repeat steps 2 and 3 for the second UV curve.
5	Select ***Operations:Divide***. *Result*: The ***Divide*** dialog box opens.

Step	Action
6	• Select the first result curve from the subtractions in the first ***Source chromatogram and curve*** list. • Select the second result curve from the subtractions in the second ***Source chromatogram and curve*** list.
7	Click the checkbox for ***Threshold*** and type values for each curve. This results in the following: • The quotient is set to 1.0 if either of the sample values is closer to zero than the threshold value. Very high quotient values are prevented if division is performed with values close to zero. Very low quotient values are also prevented. ***Note:*** *Default* ***Threshold*** *values are entered by UNICORN. The values can be changed.*
8	Click ***OK***. *Result*: A new curve is added to the chromatogram in the first available position. By default, the curve name will have a ***DIV*** ending.

Filter the result curve

The resulting curve can be filtered to reduce noise and to remove ghost peaks. The table below describes how to filter the curve.

Step	Action
1	Select ***Operations:Smooth***. Result: The ***Smooth*** dialog opens.
2	• Select the ***Source Chromatogram***. • Select a ***Filter Type***. **TIP:** The ***Median*** filter is recommended to remove noise that appears as spikes or occurs in a small area of the curve. • Click **OK**. *Result*: A new curve is added to the chromatogram in the first available position. By default, the curve name will have a ***SMTH*** ending.

4.5 Compare different runs

Introduction

This section describes

- how to make comparisons between curves or chromatograms from different runs
- how to present curves or chromatograms from different runs
- how to compare curve parameters among curves from different runs
- how to view several chromatograms at the same time
- how to overlay curves from different runs in one chromatogram
- how to stack curves from different runs in one chromatogram
- how to create mirror images

In this section

This section contains these sub-sections:

Section	See page
4.5.1 Open and compare chromatograms	160
4.5.2 Open and compare curves	167
4.5.3 Shift curves	176
4.5.4 Create a mirror image	177

4.5.1 Open and compare chromatograms

Introduction

This section describes

- how to import chromatograms from other result files to an open result file, and
- how to compare chromatograms in a result file.

Commands to use

Two commands in the ***Evaluation*** module can be used to import chromatograms from result files into an open result file:

- ***File:Open to compare***

  This is the preferred option when you search for many chromatograms in a *specific* folder based on defined selection criteria. See "How to import chromatograms with the command ***File:Open to compare***" below.

- ***File:Open***

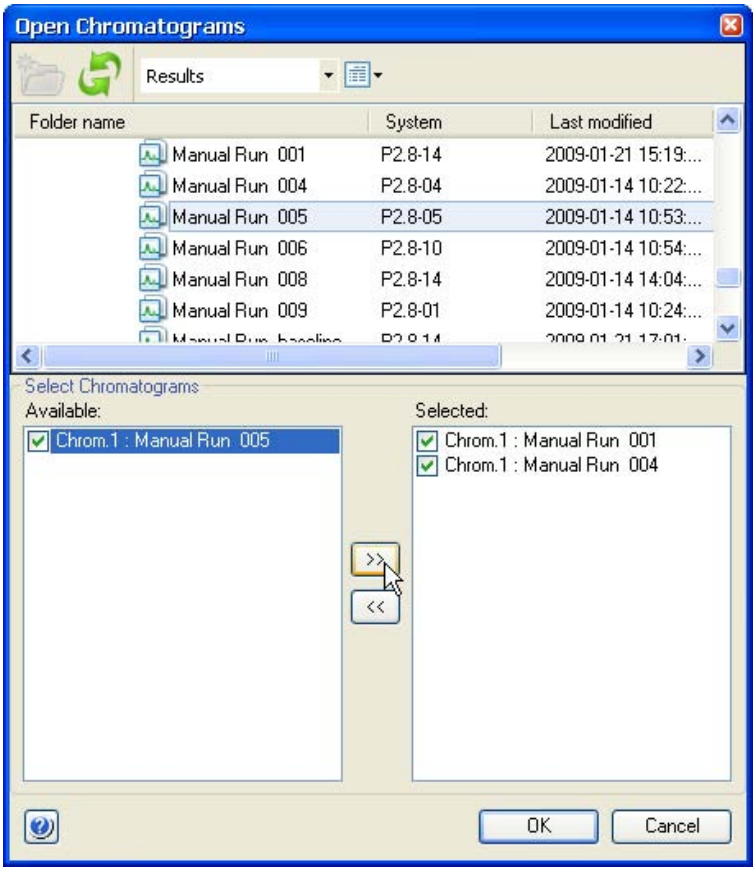
  This is the preferred option to import any individual chromatograms from result files in *different* folders. See "How to import chromatograms with the command ***File:Open***" below.

Open chromatograms

You can display several chromatograms, from different results, simultaneously for comparison. The table below describes how:

Step	Action
1	Open the result containing the first chromatogram.

Step	Action
2	• Choose the ***File:Open*** menu • Choose the ***Chromatogram*** menu item *Result*: The ***Open Chromatograms*** dialog opens.



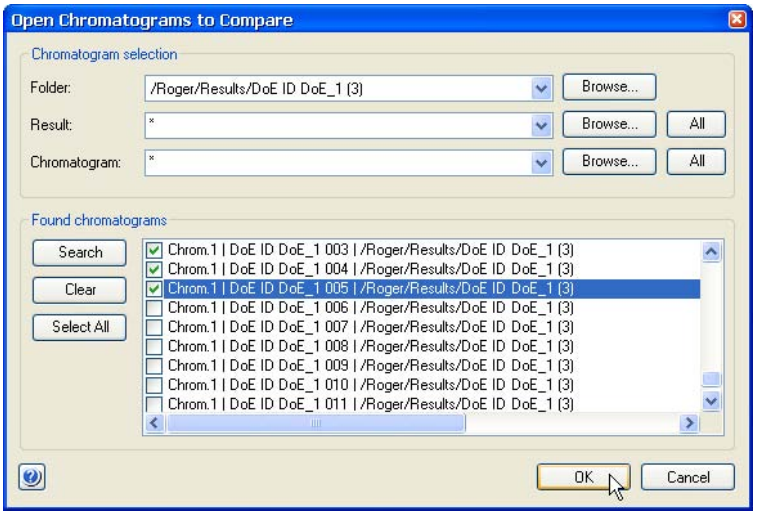
3	Select a result in the browser pane. *Result*: The chromatograms in the result are listed in the ***Available*** list.

Step	Action
4	• Click the checkbox for each chromatogram in the ***Available*** list that you want to select. • Click the selection button (as illustrated above). *Result*: The chromatogram is added to the ***Selected*** list. **TIP:** You can click the de-selection button to remove selected chromatograms from the list. This will remove all chromatograms with checkmarks. Note that you must first click to remove the checkmark from each chromatogram that you want to keep in the ***Selected*** list.
5	• Repeat step 4 until you have selected all the chromatograms you want to open. • Click the ***OK*** button. *Result*: The selected chromatograms open in the ***Evaluation*** module. ***Note:*** *If the names of the imported chromatograms already are used they will be sequentially numbered for identification purposes. Each chromatogram will be opened in its own tab. However, depending on the number of chromatograms and the length of the names, not all tabs may be visible at once. Click the arrow symbol on the top right corner of the chromatogram pane to show a list of all open chromatograms. You may select the chromatogram you want to view from this list.*

Import chromatograms with the command File:Open to compare

The table below describes how to import chromatograms with the ***File:Open to compare*** command. The search is performed at specific locations or with specific search criteria. This method is useful if, for example, you want to import chromatograms from all files of a scouting folder.

Step	Action
1	Choose ***File:Open to compare:Chromatograms*** in the ***Evaluation*** module. *Result*: The ***Open Chromatogram to Compare*** dialog box is displayed.


Open Chromatograms to Compare
Chromatogram selection
Folder:
/Roger/Results/DoE ID DoE_1 (3)
Browse...
Result:
*
Browse...
All
Chromatogram:
*
Browse...
All
Found chromatograms
Search
Clear
Select All
Chrom.1 | DoE ID DoE_1 003 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 004 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 005 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 006 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 007 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 008 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 009 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 010 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 011 | /Roger/Results/DoE ID DoE_1 (3)
OK
Cancel

2

- Click the ***Browse*** button beside the ***Folder*** droplist

  This opens a browse dialog where you can select the result folder where you want to search for chromatograms.

  Note: *The search will not include the contents of subfolders. You must select the subfolders individually for the search.*

- Repeat this step to define specific results or chromatograms if desired.

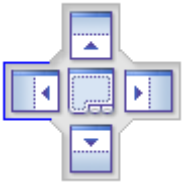
TIP: Click the droplist arrow buttons to view searchpaths that you have used previously. You can click selections in these lists to search the same locations again.

Step	Action
3	• Click the ***Search*** button in the ***Found chromatograms*** field and a list of chromatograms will be displayed based on the designated search criteria. • A new search can be performed with new search criteria without erasing the initial chromatograms from the list. • Select the chromatograms that you want to import. If you click the ***Select All*** button, all the displayed chromatograms will be imported. • If you want to clear the list of displayed chromatograms, click the ***Clear*** button.
4	• Click ***OK***. *Result*: All the selected chromatograms are opened in the ***Evaluation*** module. ***Note:*** *If the names of the imported chromatograms already are used they will be sequentially numbered for identification purposes. Each chromatogram will be opened in its own tab. However, depending on the number of chromatograms and the length of the names, not all tabs may be visible at once. Click the arrow symbol on the top right corner of the chromatogram pane to show a list of all open chromatograms. You may select the chromatogram you want to view from this list.*

Display and compare the imported chromatograms

By default, the chromatograms are opened behind each other in the full chromatogram pane. The table below describes how to arrange the imported chromatograms so that several can be viewed and compared at the same time:

Arrange the chromatograms

Step	Action
1	Click the tab of one of the chromatograms and hold the mouse button pressed. *Result*: A positioning icon is displayed in the chromatogram pane. 
2	Move the mouse pointer to the area of the window where you want to place the pane.
3	The arrows of the symbol show the side of the pane where the selected chromatogram will be docked. Drag the mouse pointer over the appropriate arrow. *Result*: The area where the chromatogram will be docked is colored.
4	Release the mouse button. *Result*: The pane is moved and docked in the new position. **TIP:** Several chromatograms may share a space, either placed on top of each other or arranged side by side or above each other. Click the center position of the symbol to place a chromatogram on top of another.

TIP:

- To restore the original layout with the chromatograms displayed as tabs, select ***View:Chromatograms as Tabs***.
- To view all chromatograms side by side, select ***View:Tile All Chromatograms***.

Display all chromatograms on the same scale

Step	Action
1	Click the ***Customize*** icon or select ***Tools:Customize*** to open the ***Customize*** dialog for any chromatogram.
2	Select the X-Axis tab and make the changes to the ***Base*** and the ***Axis scale***.
3	Select the ***Apply to all chromatograms*** option and click ***OK***.

Note: *Imported chromatograms cannot be shown with column volume as the X-axis base.*

4.5.2 Open and compare curves

Introduction

This section describes how to import or copy curves from different runs into one chromatogram for comparison.

Commands to use

Two commands can be used to import curves from result files into one chromatogram:

- ***File:Open to compare***

  This is the preferred option if you want to automatically search result files that are stored in the same folder to locate all curves of a specified type, for example, all UV curves. This is especially useful for comparison of curves from scouting runs. Moreover, the imported curves can be automatically overlaid, stacked or presented as mirror images. See "File:Open to compare" below.

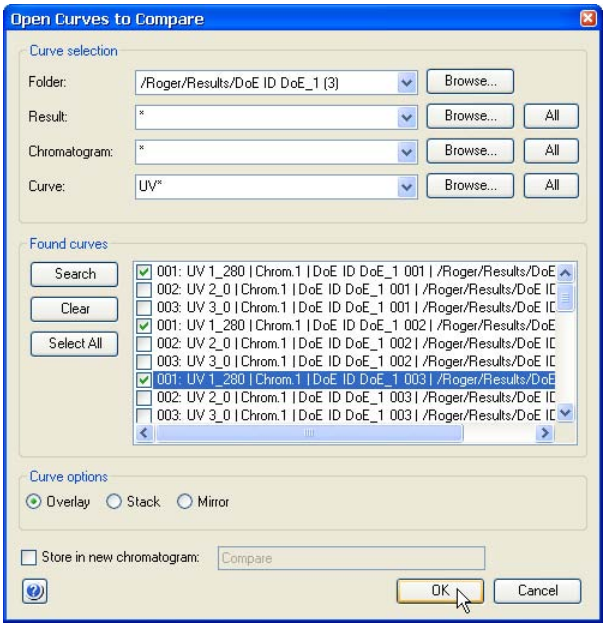
- ***File:Open:Curves***

  This is the preferred option to import individual curves. See "File:Open:Curves" below.

Note: *Original curves are underlined in the chromatogram, imported and created curves are not underlined.*

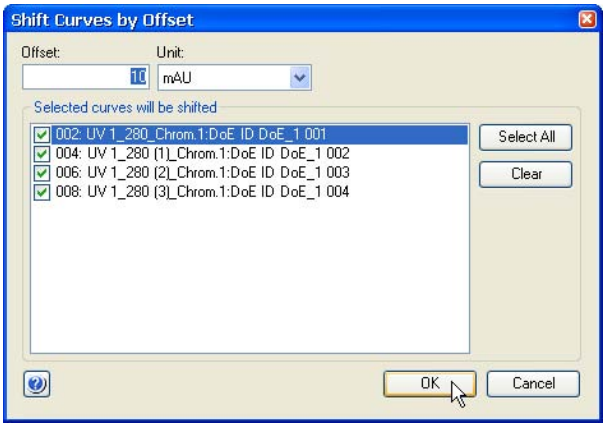
File:Open to Compare

The table below describes how to import curves to a chromatogram with the command ***File:Open to Compare***:

Step	Action
1	In the ***Evaluation*** module, • choose ***File:Open to compare:Curves*** *or* • click the ***Open curves to compare*** toolbar button. *Result*: The ***Open Curves to Compare*** dialog opens. 

Step	Action
2	• Select the desired search criteria in the ***Folder***, ***Result***, ***Chromatogram*** and ***Curve*** droplists of the ***Curve selection*** section. • Click ***Search*** and a list of curves will be displayed based on the selected search criteria. **TIP:** A new search can be performed with new search criteria without erasing curves located in the previous search. • Select the check boxes for the curves that you want to import. Click the ***Select All*** button if you want to import all the curves. • If you select the ***Store in new chromatogram*** option, the curves will be imported into a new chromatogram. This is recommended to keep the source chromatogram free of too many additional curves.
3	• Select how to display the imported curves in the ***Curve options*** field. The options are described in "*Curve options*" below. • Click ***OK***.

Step	Action
4	If you selected the ***Stack*** option in step 3, the ***Shift Curves by Offset*** dialog is displayed:



- You can set the ***Offset*** value to increase or decrease the offset distance between the curves.
- Click ***OK***.

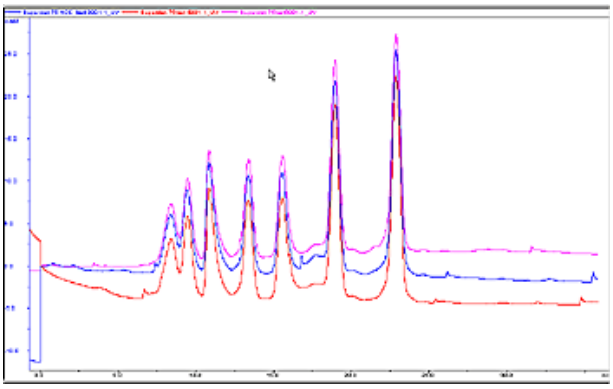
Result: Depending on your previous choices, the imported curves are now displayed in the source chromatogram or in a newly created chromatogram.

Note: *If curves with several different units have been selected, the curves with each different unit will be grouped together with separate offset from the other groups.*

Curve options

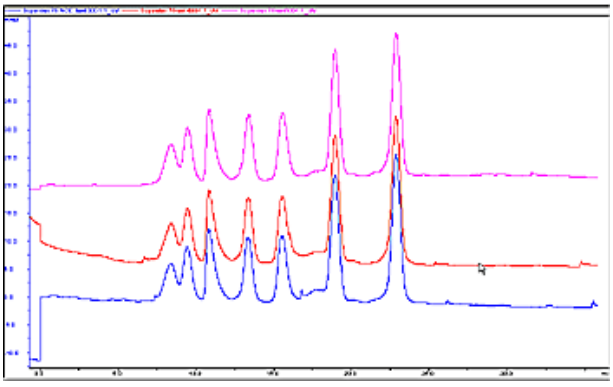
The illustrations below show the different curve presentation options:

Overlay



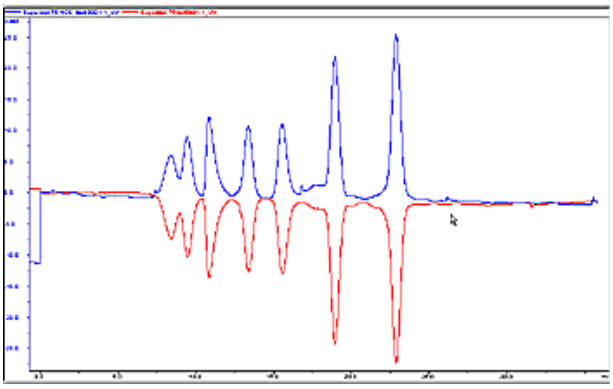
The curves are presented overlaid on one another.

Stack



The curves are presented with a given offset Y-axis value so that the curves are stacked and distinct from one another.

Mirror



For comparison of *two* imported curves. One curve is inverted in the Y-axis and thus appears to mirror the other curve.

Change comparison settings

The table below describes how to change other comparison settings:

Step	Action
1	Click the ***Customize*** icon to open the ***Customize*** dialog.
2	Select or de-select the check boxes on the ***Curve*** tab to compare a different set of curves.
3	• Select the ***Y-Axis*** tab to change the curve scales either - individually or - all with the same scale (click the ***All With This Unit*** button). • Click ***OK*** to update the curves.

Step	Action
4	• If you stacked the curves and want to change the stack offset - choose ***Operations:Shift offset*** and - type a new ***Offset*** value. • Click ***OK*** to close the dialog and update the curves. **TIP:** The individual curves can also be moved (see *Section 4.5.3 Shift curves, on page 176*).

File:Open:Curves

The table below describes how to import individual curves into an active chromatogram with the ***File:Open:Curves*** command:

Step	Action
1	Make sure that the destination chromatogram for the imported curve(s) is active on the screen. • Select ***File:Open:Curves*** in the ***Evaluation*** module. *Result*: The ***Open Curves*** dialog is displayed.
2	• Select the folder and the result file in the browse field of the dialog. • Select a chromatogram on the ***Available curves*** droplist. Usually there is just one chromatogram. *Result*: The available curves are listed below. • Click the checkboxes on the list for the curves that you want to import and click the select (right arrow) button. *Result*: The selected curve(s) is displayed in the ***Selected curves*** list. **TIP:** To remove a curve from the ***Selected curves*** list, ensure that the corresponding checkbox is selected and that the checkboxes for all other curves are unselected, and then click the de-select (left arrow) button.
3	• Repeat step 2 if you want to import curves from other chromatograms. • Click ***OK*** when you have selected the curves you want. *Result*: The selected curves are added to the active chromatogram.

Copy curves into a new chromatogram

A practical way to compare curves is to create a chromatogram and copy curves from different chromatograms into the new chromatogram. The comparisons are then performed in the new chromatogram.

The table below describes how to copy curves into a chromatogram:

Step	Action
1	• Choose ***File:New:Chromatogram***. Result: The ***New Chromatogram*** dialog is displayed. • Type a name for the chromatogram and click ***OK*** to add the empty chromatogram to the current result.
2	**Open the source chromatogram(s)** Choose ***File:Open:Chromatogram*** to open the chromatogram(s) that contains the curves you want to copy. *Result*: The ***Open Chromatogram*** dialog opens.
3	• Select the result file. • Click the check box for the source chromatogram in the ***Available*** list. • Click the select (right arrow) button. • Click ***OK***. *Result*: The source chromatogram opens.
4	**Copy the curves** • Choose ***Edit:Copy Between Chromatograms:Curves***. *Result*: The ***Copy Curve*** dialog is displayed.

Step	Action
5	• Select the source chromatogram and a curve of interest in the ***Source Chromatogram*** field. • Select the new chromatogram and a curve position in the ***Target Chromatogram*** field. A default curve name with the ending ***COPY*** is suggested in the ***Curve name*** field. • Click the ***Copy*** button. • Repeat this step for as many curves as you want, from the same or other chromatograms. **TIP:** You can open more source chromatograms with the ***File:Open:Chromatogram*** command. • Click the ***Close*** button when you have copied all curves.

Alternative way to copy curves

An alternative way to copy curves into one chromatogram is to

- create a new chromatogram by copying an existing chromatogram and saving it under a new name
- import more curves into the new chromatogram according to the instructions described above in this section.

4.5.3 Shift curves

Introduction

You can stack and stretch curves from different runs to better visualize the differences. If you want to position a curve with precision, the ***Shift*** function should be used. Each curve is repositioned by a precise value and the instruction is logged in the evaluation log.

Note: *The **Shift** function requires the curves to be present in one chromatogram.*

Move a curve with the Shift function

The table below describes how to use the ***Shift*** function:

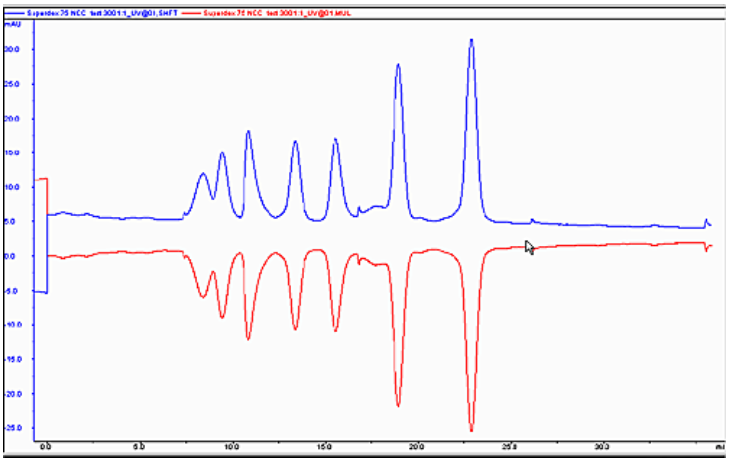
Step	Action
1	• Make sure that a chromatogram with the relevant curves is open in the ***Evaluation*** module. • Choose ***Operations:Shift***. *Result*: The ***Shift*** dialog is displayed.
2	• Select the curve to be shifted in the ***Source chromatogram and curve*** list. • Select a curve position in the ***Target chromatogram and curve*** list. • Type a new ***Curve name*** or accept the default. The default curve name ending is ***SHFT***.
3	• Select the axis/axes along which the shift is to be made: - along the X-axis (***Shift retention***) - along the Y-axis (***Shift amplitude***). • Type the shift value(s). • Click ***OK***.
4	Repeat steps 1 to 3 to shift other curves in the chromatogram.

4.5.4 Create a mirror image

Instruction

A very useful way to compare the features of two curves is to produce a mirror image of one curve. The table below describes how to do this:

Step	Action
1	• Open a chromatogram to insert the relevant curves into. • Choose ***File:Open to Compare:Curves***. *Result*: The ***Open Curves to Compare*** dialog is displayed.
2	• Select the desired search criteria in the ***Folder***, ***Result***, ***Chromatogram*** and ***Curve*** droplists of the ***Curve selection*** section. • Click ***Search*** and a list of found curves will be displayed based on the selected search criteria. **TIP:** A new search can be performed with new search criteria without erasing curves located in the previous search.
3	• Select the checkboxes for the curves that you want to import. • Select ***Mirror*** in the ***Curve options*** field. • Click ***OK***. *Result*: The curves are displayed with one of the curves as a mirror image in the active chromatogram window.

Step	Action
4	**Shift the mirror image curve downwards** Shift the mirror image curve downwards for an improved presentation: • Choose ***Operations:Shift***. *Result*: The ***Shift*** dialog box is displayed. • Select the curve to be shifted in the ***Source chromatogram and curve*** list. • Select the same curve number in the ***Target chromatogram and curve*** list box as in step 2. • Select the ***Shift amplitude*** checkbox since the shift is to be made along the Y-axis. • Type a shift value. • Click ***OK***. The illustration below shows a curve and a mirror image curve displayed. 

4.6 The Multi Result Peak Compare wizard

Introduction

This section describes how to use the ***Multi Result Peak Compare*** wizard to make statistical comparisons between peaks in different results, for example by comparing area, retention, etc. The calculated statistics can be presented graphically or in a spreadsheet.

Note: *Before you proceed with the wizard comparison, all results must be peak integrated using the same retention unit.*

Wizard workflow

The pages of the wizard are described in the table below:

Page	Description
Entry dialog	Welcome page.
Retention unit selection	Select the retention unit that was used previously during peak integration.
Data selection	Select the curves for comparison.
Peak selection	Identify the peaks for comparison in all selected curves. • Select peaks in the first curve • The system identifies the corresponding peaks in all other selected curves.
Peak data selection	Select peak characteristics to be included in the comparison result.
Data view	View the results of the comparison.

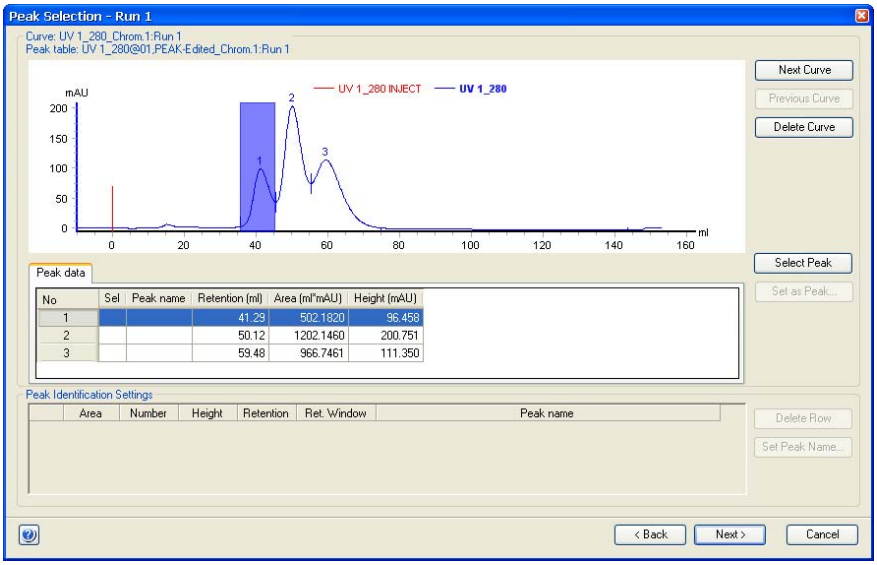
Step 1: Select the retention unit

The table below describes how to select the retention unit:

Step	Action
1	In the ***Evaluation*** module, • choose ***File:Multi Result Peak Compare*** or • click the ***Multi Result Peak Compare*** toolbar icon *Result*: The ***Multi Result Peak Compare*** wizard entry dialog is displayed. **TIP:** At this point you may choose to use stored wizard settings. This is described in "Open the stored wizard settings" below.
2	Click the ***Next*** button to display the ***Retention Unit Selection*** dialog.
3	Select the retention unit that was used when the compared results were integrated: • ***min*** • ***l*** • ***ml*** • ***µl*** • ***nl*** Click the ***Next*** button to proceed to the ***Data Selection*** dialog.

The Data Selection dialog

The illustration below shows the ***Data Selection*** dialog.



Step 2: Select curves to compare

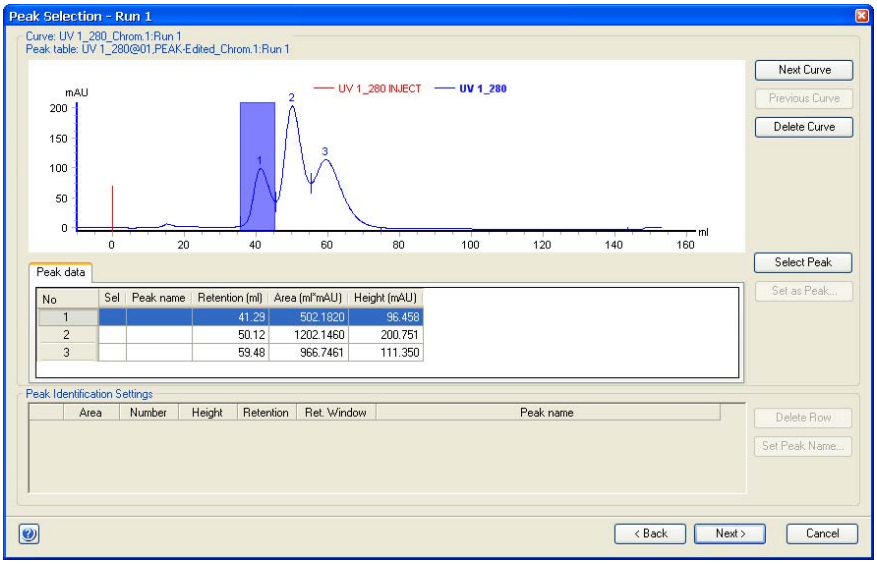
The table below describes how to select curves to compare:

Step	Action
1	Use the droplists and ***Browse*** buttons in the ***Curve selection*** field to specify the result files, chromatograms and curves for comparison. **TIP:** Click the ***All*** button if you want to select all available results, chromatograms or curves.
2	Click the ***Search*** button in the ***Found curves*** field. *Result*: A list of all curves that matched the search criteria is displayed in the ***Found curves*** field.

Step	Action
3	• Select the checkboxes (or click the ***Select All*** button) of the desired curves within the ***Found curves*** field. • Click the **Next** button to proceed to the ***Peak Selection*** dialog. ***Note:*** *If any of the results was not peak integrated before, or if different retention units were used, you will be asked if you wish to proceed only with the results that were integrated with the selected retention unit, or if you want to cancel the wizard.*

The Peak Selection dialog

The illustration below displays the ***Peak Selection*** dialog:



Dialog description

The dialog displays the following properties for the first of the chosen curves:

- The integrated peaks and the associated peak table in ***Peak data***.
- The ***Peak Identification Settings*** table. Its purpose is to identify the peak parameter to be used in the comparison.

Adjust improper peak integrations

The table below describes what to do if the peaks in the curve window do not appear to be integrated properly (for example if ghost peaks are numerically labelled).

Step	Action
1	Click the ***Cancel*** button to quit the wizard.
2	Perform a peak integration (see *Section 4.2 Peak integration, on page 98*) and verify that the resulting curve is properly integrated.
3	Repeat the ***Multi Result Peak Compare*** wizard operation.

Step 3: Select the peaks

All peaks identified during the peak integration are numerically labelled. In this step the peaks of interest are selected in the ***Peak Selection*** dialog, first manually in one curve, then automatically in all selected curves.

Select the peaks in the first curve

- Double-click a peak in the curve window or click the peak once and then click the ***Select peak*** button.

  Result: The peak is assigned an identification letter (A, B, C....) in ***Sel*** column in the ***Peak data*** table and under the peak in the Curves window. A row with the peak parameters is added in the ***Peak Identification Settings***. The order of the letter is determined by the order of the selection.

- Repeat the selection for all peaks of interest in the curve.

TIP: Click and drag in the curve window to zoom into selected peaks to simplify accurate peak identification. Right-click and click the ***Reset Zoom*** button to reset the zoom to the full view.

Select identification criterion for each peak in the first curve

Set the desired peak identification criterion:

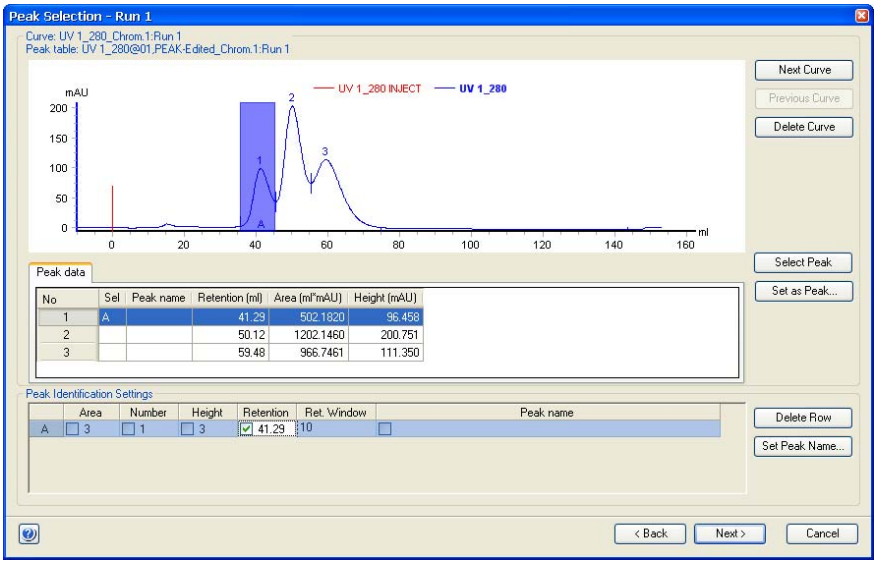
- Click the desired parameter in the ***Peak Identification Settings*** table. For example, if you have selected the highest peak in the curve and want to compare the highest peak among all curves, select the ***Height*** check box. The available parameters are:
  - Area: Same relative area size.
  - Number: Same number in order after elution.
  - Height: Same relative height.

- Retention: Same retention value.
- Peak name: Same peak name.

- Repeat the selection for all peaks of interest in the first curve.

Result: Based on this selection, UNICORN locates the corresponding peaks in all selected curves and assigns the same identification letters.

In the illustration below, only the first peak has been selected and has therefore been assigned the identification letter ***A***. It is the third highest peak and has therefore ***3*** as ***Height*** in the ***Peak Identification Settings*** table. ***Retention*** has been selected as identification criteria:



Verify the peak identification in all selected curves

Use the ***Next curve*** and ***Previous curve*** buttons to navigate forward and backward among your selected curves and manually check the peak identification made by the software. Verify that UNICORN has identified the correct peaks in the ***Peak Identification Settings*** table and/or curves window of each curve.

If UNICORN has identified other peaks than the intended ones, you can change the peak identification manually according to the table below:

If you want to...	then...
remove a peak identification	• click the desired peak in the curves window • click the ***Set as peak*** button • choose ***None*** in the ***Set As Peak*** dialog • click ***OK***. Result: The peak in this curve will not be included in the statistical calculation. The peak identification letter is removed from the curves window.
replace or add a peak identification	• click a peak in the curves window • click the ***Set as peak*** button • choose a letter in the ***Set As Peak*** dialog • click ***OK***. Result: The peak identification letter is replaced/added in the curves window and ***Peak data*** table, and the row is edited/added in the ***Peak Identification Settings*** table.
remove a row from the table	• select the row • click the ***Delete row*** button. Result: All peaks with the same identification letter in the selected curves are excluded from the statistical calculation. The peak identification letter is removed from the curves window and ***Peak data*** table, and the row is removed from the ***Peak Identification Settings*** table. ***Note:*** *If you click **Delete row** without first selecting a row, the first row (A) is deleted by default.*

Other possible actions you can perform

If desired, you can assign a name to a chosen peak:

- Click the peak label of the row, for example ***A***.
- Click the ***Set peak name*** button.
- Type a new name and click ***OK***.

  Result: All peaks labeled ***A*** in the selected curves are assigned the name.

TIP: This can be useful when you compare multiple peak parameters and you wish to have peak names other than “Peak A”, “Peak B”, etc., to simplify evaluation of the comparison.

If desired, you can change the choice of curves.

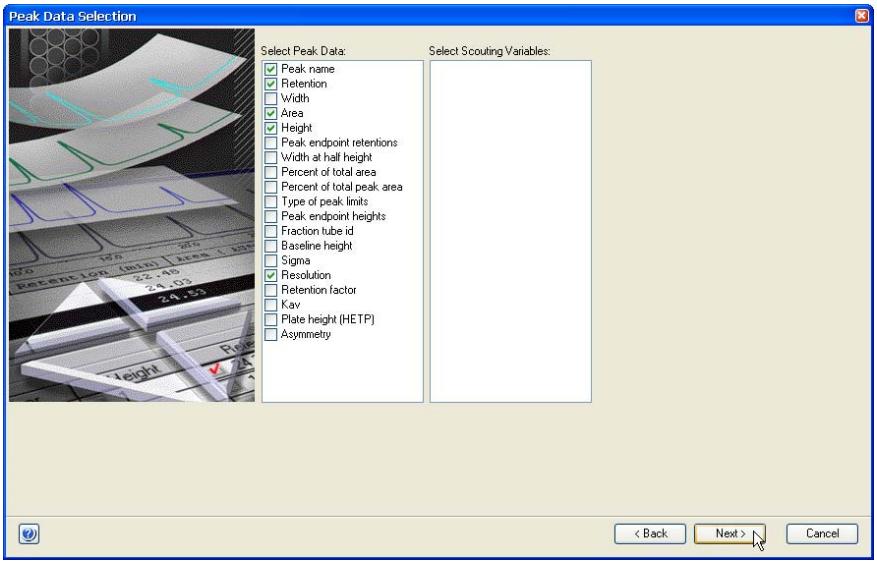
- Click the ***Delete curve*** button to delete a curve from the comparison, if it does not prove useful for your comparison.
- Click the ***Back*** button to navigate back to the ***Data Selection*** dialog to add new curves to your comparison.

Peak selection completion

- Verify the identification of the corresponding peaks in the selected curves again, if necessary.
- When all peak selections and identification settings are complete, click the ***Next*** button to proceed to the ***Peak Data Selection*** dialog.

Step 4: Select the Peak Data

The illustration below displays the ***Peak Data Selection*** dialog:

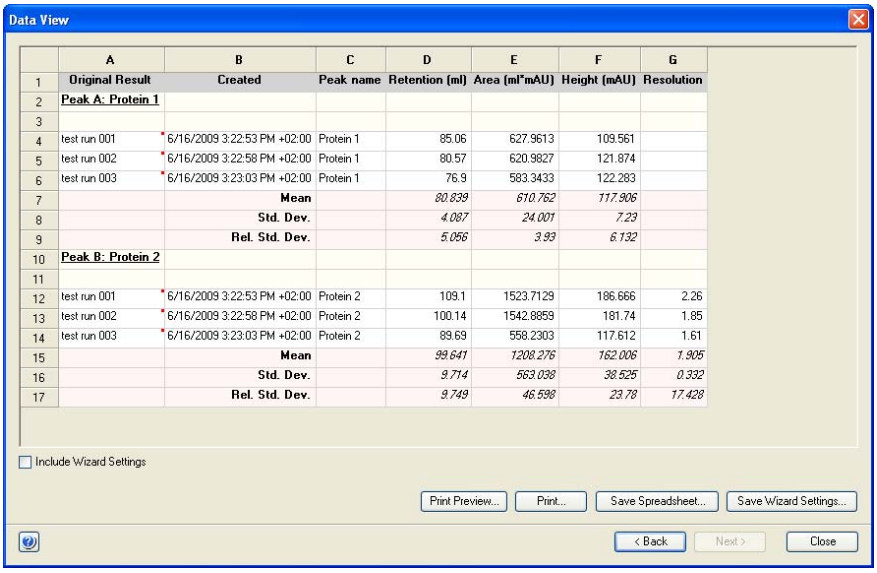


The table below describes how to select the peak data:

Step	Action
1	• In the ***Select Peak Data*** list, select the peak characteristics on the list that you want to include in your comparisons. • If available, select the appropriate ***Scouting*** variables.
2	• Click the ***Next*** button and proceed to "Step 5: The ***Data View*** dialog" below.

Step 5: The Data View dialog

The ***Data View*** dialog presents a statistical comparison of the chosen data for the designated peak comparisons. The illustration below shows the dialog. In this example, there are three curves and two identified peaks. Peak A has been named ***Protein 1***, and peak B named ***Protein 2***.



	A	B	C	D	E	F	G
1	Original Result	Created	Peak name	Retention (ml)	Area (ml*mAU)	Height (mAU)	Resolution
2	Peak A: Protein 1						
3							
4	test run 001	6/16/2009 3:22:53 PM +02:00	Protein 1	85.06	627.9613	109.561	
5	test run 002	6/16/2009 3:22:58 PM +02:00	Protein 1	80.57	620.9827	121.874	
6	test run 003	6/16/2009 3:23:03 PM +02:00	Protein 1	76.9	583.3433	122.283	
7		Mean		*80.839*	*610.762*	*117.906*	
8		Std. Dev.		*4.087*	*24.001*	*7.23*	
9		Rel. Std. Dev.		*5.056*	*3.93*	*6.132*	
10	Peak B: Protein 2						
11							
12	test run 001	6/16/2009 3:22:53 PM +02:00	Protein 2	109.1	1523.7129	186.666	2.26
13	test run 002	6/16/2009 3:22:58 PM +02:00	Protein 2	100.14	1542.8859	181.74	1.85
14	test run 003	6/16/2009 3:23:03 PM +02:00	Protein 2	89.69	558.2303	117.612	1.61
15		Mean		*99.641*	*1208.276*	*162.006*	*1.905*
16		Std. Dev.		*9.714*	*563.038*	*38.525*	*0.332*
17		Rel. Std. Dev.		*9.749*	*46.598*	*23.78*	*17.428*

The table below describes how to use the command buttons of the dialog:

Command button	Function
Print Preview	Opens a preview dialog showing what the ***Data View*** spreadsheet will look like when printed.
Print	Prints the spreadsheet.

Command button	Function
Save Spreadsheet	Allows you to save the result from the multi result peak comparison as: • an Excel file (.xls) or • Tabbed text (.txt)
Save Wizard Settings	See "Save the Wizard Settings" below.
Close	Ends the ***Multi Result Peak Compare*** wizard.

Save the Wizard Settings

The settings from the comparison can be saved, in order to use the same settings on other results. The table below describes how to save the wizard settings:

Step	Action
1	• Click the ***Save Wizard Settings*** button. *Result*: The ***Save Wizard Settings*** dialog opens.
2	Type a name in the ***Wizard settings name*** field.
3	• If the settings are to be used by all users on the system, select the ***Global wizard settings*** checkbox. • Click ***OK***. • Click ***Close*** to close the wizard. **TIP:** The ***Global wizard settings*** checkbox can also be used to toggle between lists of stored global and stored user settings.

Open the stored wizard settings

The table below describes how to open the stored wizard settings:

Step	Action
1	• Open the Multi Result Peak Compare wizard and click the ***Select Stored Wizard Setting*** button. *Result*: The ***Select Wizard Settings*** dialog opens.
2	• Select the desired saved settings from the list. • Click ***OK***. *Result*: The ***Multi Result Peak Compare*** wizard opens with the saved settings. **TIP:** The ***Global Wizard Settings*** checkbox is used to toggle between lists of stored global and stored user settings.

4.7 Rename folders, results, chromatograms, curves and peak tables

Rename folders and files

Folders and files may be renamed in the ***Result Navigator*** either by

- right-clicking the object and choosing ***Rename*** from the shortcut menu

  or

- choosing ***Edit:Rename:Navigator Object***

This will highlight the name of the selected object and you can type a new name instead.

Note: *You cannot rename assigned home folders.*

Rename chromatograms, curves and peak tables

The table below describes how to rename chromatograms, curves or peak tables in the ***Evaluation*** module.

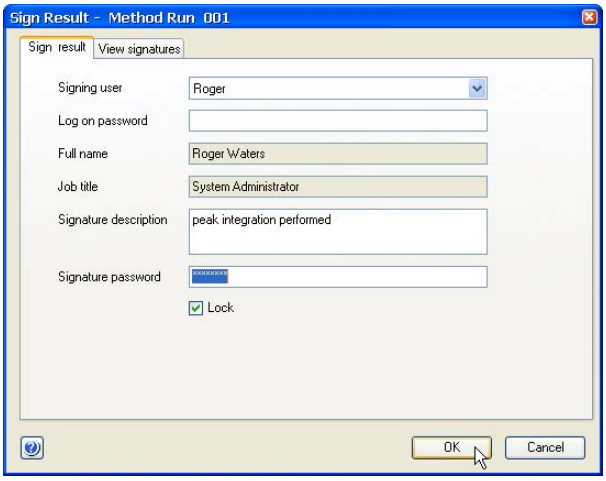
Step	Action
1	Choose ***Edit:Rename*** and the relevant option ***Chromatogram***, ***Curve*** or ***Peak Table***. *Result*: The ***Rename*** dialog opens.
2	• Select the appropriate object. • Type a new name in the ***Name*** field. • Click ***OK***.

Note: *The original chromatogram or original raw data curves cannot be renamed. They will not be available in the* ***Rename*** *dialog.*

4.8 Sign results electronically

Instruction

Result files can be signed electronically to enhance data file security. The table below describes how to sign a result file electronically in the ***Evaluation*** module:

Step	Action
1	Choose ***File:Sign Result***. *Result*: The ***Sign Result*** dialog opens. 
2	The ***Sign result*** tab shows the properties for the current user. You can also choose another user from the droplist. If you choose a new user, the corresponding password must be typed in the ***Log on password*** text box. • Type your log on password in the ***Log on password*** field. • Type a short text description for the signed operation in the ***Signature description*** field (e.g. "peak integration performed"). The ***Lock*** check box is selected as default, to lock the result file from further changes. ***Note:*** *You should only lock the result when you are sure that the result file will not be modified anymore.*
3	• Type your signature password in the ***Signature password*** field. • Click ***OK***.

Signatures associated with the result

The ***View Signatures*** tab of the ***Sign Result*** dialog provides a list of all signatures associated with the current result. The information on this tab is for viewing purposes only and cannot be changed.

4.9 Save results

Introduction

After you have finished the evaluation process, you can save all the changes you have made to the chromatograms, including newly created curves and chromatograms that you have imported and created.

Delete unwanted curves

All the curves that you created during your manipulations will be saved in the chromatogram. If some of these curves are no longer needed, select ***Edit:Delete:Curves*** in the ***Evaluation*** module to remove the curves.

Note: *The original curves that were created during the run can never be deleted.*

Save the results

You can either save your edited results in the original file or in a new result file. The table below describes how to save the results in the ***Evaluation*** module.

If you want to save the edited results...	then...
in the original result file	• select ***File:Save***. or • click the ***Save*** toolbar icon.
in a new result file	• select ***File:Save as***.

Note: *The previous version of the result file will be overwritten if you save the changes. This cannot be reversed. However, the raw data curves remain unchanged.*

Exit the Evaluation module

The table below describes how to exit the ***Evaluation*** module:

If you want to...	then...
close only the ***Evaluation*** module	click the red cross icon in the top right corner of the module window.
exit UNICORN	choose ***File:Exit UNICORN***.

Note: *If there are unsaved changes, a dialog box opens with an option to save the changes before exit.*

5 Import and export results

Introduction

Curves and data can be imported and exported in different formats. This chapter describes how to import and export results.

In this chapter

This chapter contains these topics:

Section	See page
5.1 Import results	196
5.2 Export results	198

5.1 Import results

Introduction

This section describes how to import curves and result data.

Import curves

Individual curves saved in ASCII format (as ***.asc*** or ***.txt*** files) may be imported into the active chromatogram. The table below describes how to import curves:

Step	Action
1	• Choose ***File:Import:Curves***. *Result*: The ***Import*** dialog box opens.
2	• Locate the file that contains the curve and click the file. • Click the ***Open*** button. *Result*: The ***Import Curves*** dialog opens, showing the available curves in the file.
3	• Select the curve(s) to import. • Click the ***OK*** button. *Result*: The curves are opened in the active chromatogram.

Note: *A curve can only be imported in the base unit that is was exported. For volume base, the curve is shown in chromatogram with ml and CV as x-axis. For time base, the curve is shown in in chromatogram with min as x-axis.*

Import results

Results that have been exported from a UNICORN database may be imported into the database again. The table below describes this:

Step	Action
1	• Choose ***File:Import:Entire Result*** *Result*: The ***Import*** dialog opens.

Step	Action
2	• Locate and click the zip-file that contains the result. To select several files, press Shift while you click the files. • Click the ***Open*** button. *Result*: The ***Import Result*** dialog opens.
3	• Select the destination folder for the result. • Click the ***Import*** button. *Result*: The result files are individually unzipped and imported into the selected folder.

Note: *A result file cannot be imported into a folder if the folder already contains a result with the same name. In that case you must rename the file.*

TIP: Result files created in UNICORN 5 using ÄKTAexplorer™ or ÄKTApurifier™ can be imported. These are saved as .res files, not .zip files. The results can be used for comparison with results created in UNICORN 6. This is described further in the *UNICORN 6 Administration and Technical Manual*.

5.2 Export results

Introduction

This section describes how to export entire results and result items, including

- curves
- documentation
- peak tables

  and
- Design of Experiment projects

The section also describes how to copy items using the Windows clipboard.

Export options

Select ***File:Export*** in the ***Evaluation*** module to export data from an open result file. The following export options are available:

If you choose...	Then the options are...
to UNICORN	• ***Curves*** or • ***Entire Result***
Design of Experiment - Modde	Export of a Design of Experiment to a MODDE Investigation Project file.
Externally	• ***Curves*** • ***Peak Table*** • ***Documentation*** or • ***Copy to Metafile***

These options are explained further below.

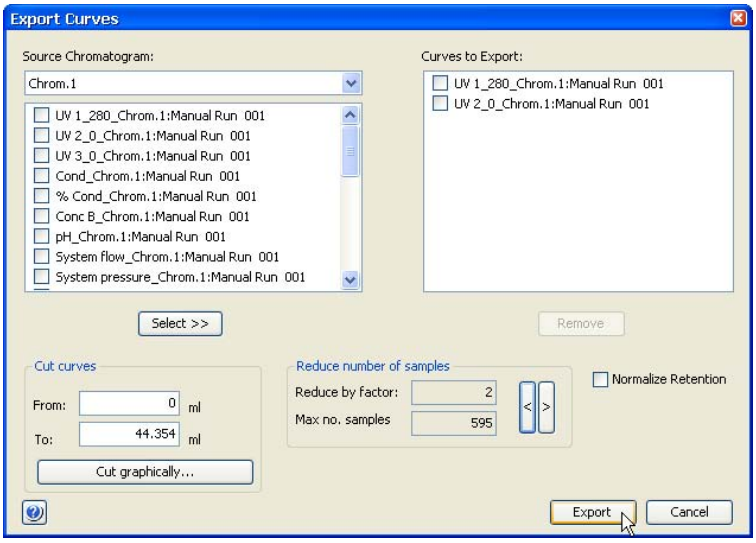
Export entire results

An or several entire result may be exported for use in another UNICORN database. The table below describes how to do this:

Step	Action
1	• Select a result in the ***Result Navigator***. To select several results, press Shift while you click the results. • Select ***File:Export:To UNICORN:Entire Result***. *Result*: The ***Export to Another UNICORN Database*** dialog opens if one result was chosen. The ***Browse For Folder*** dialog opens if several results were chosen.
2	Result files are exported as compressed ***.zip*** files only. • Select an export location. Make a new folder if desired. • Type a name in the ***File name*** field in the ***Export to Another UNICORN Database*** dialog if one result was chosen. ***Note:*** *As the results are exported individually, it is not applicable to enter a destination file name if several results are selected for export*.
3	• Click ***Save*** in the ***Export to Another UNICORN Database*** dialog or OK in the ***Browse For Folder*** dialog. *Result*: The result is saved as a zip file. If several results were exported, each result is saved as an individual zip file.

Export curves

The table below describes how to export curves in the ***Evaluation*** module.

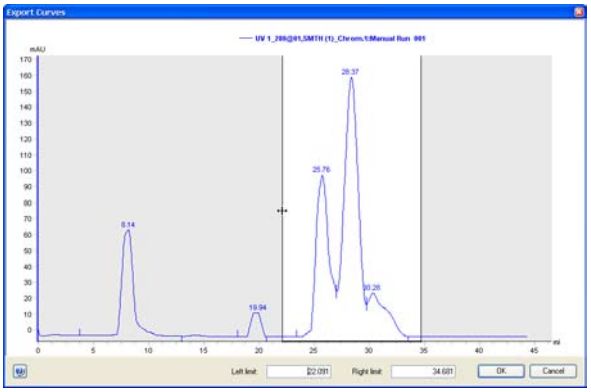
Step	Action
1	• Choose ***File:Export:To UNICORN:Curves***. or • choose ***File:Export:Externally:Curves*** *Result*: The ***Export Curves*** dialog opens. 
2	• Select the curve(s) you want to export. • Enter parameters to limit the curve(s) if necessary (described in "Limit the exported curves" below). • Click the ***Select >>*** button. • Repeat this step to select more curves.
3	Click the ***Export*** button. *Result*: The ***Export Curves to File*** dialog opens.

Step	Action
4	Select the export file format from the ***Save as type*** droplist. • ***ASCII files (*.asc)*** or • ***Comma Separated Values (*.csv)***
5	• Select a destination folder. • Type a file name and click ***Save***.

Note: *Curves are exported as series of numerical coordinates that refers to the time/volume and signal respectively.*

Limit the exported curves

You can optimize the exported curves to only the parts that you want to focus on, in the ***Export Curves*** dialog. The table below describes how to use these editing options.

Option	Instruction
Cut curves	Enter retention values in the text boxes to limit the curve to only a portion of the original curve.
Cut graphically	This button opens a graphical***Export Curves*** dialog. Move the vertical markers to the correct cutoff points. 
Reduce number of samples	Adjust the factor value or the maximum number of samples. To reduce the number of samples by a factor of five means that only every fifth point will be sampled for export.

Option	Instruction
Normalize retention	Select the ***Normalize retention*** checkbox to have all exported curves normalized to a common X-axis.

Export peak tables

The table below describes how to export peak tables.

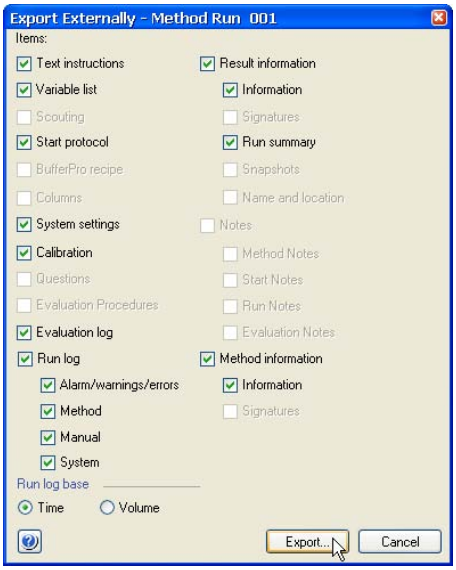
Step	Action
1	Choose ***File:Export:Externally:Peak Table***. *Result*: The ***Export Peak Table*** dialog opens.
2	• Select the source chromatogram and the peak table you want to export. • Click the ***Export*** button. *Result*: The ***Export Peak Table To File*** dialog opens.
3	Select the export file format from the ***Save as type*** droplist: • ***ASCII files (*.asc)*** • ***Excel files (*.xls)*** • ***XML files (*.xml)***
4	• Select a destination folder. • Type a file name. • Click ***Save***.

Note: *Peak tables are exported as text strings in ASCII or XML format and numerical values in the Excel format. All possible columns in the peak table are exported.*

Export documentation

The table below describes how to export the run documentation:

Step	Action
1	• Choose ***File:Export:Externally:Documentation***. *Result*: The ***Export Peak Table*** dialog opens.
2	• Select the report items you want to include in the exported documentation. **TIP:** Sub-menu items are available only if the top item is selected. For example, you must select ***Method Information*** first to be able to select ***Information*** and ***Signatures***.
3	• Click the ***Export*** button. *Result*: The ***Export Documentation to File*** dialog opens.
4	Select the export file format from the ***Save as type*** droplist: • ***ASCII files (*.asc)*** • ***Excel files (*.xls)***



Step	Action
5	• Select a destination folder. • Type a file name. • Click ***Save***.

Export the chromatogram to a Windows meta file

You can export an image of the open chromatogram as a Windows enhanced metafile. This image format can be used in other applications, for example *Microsoft Word* or *PowerPoint*. The table below describes this export:

Step	Action
1	• Choose ***File:Export:Externally:Copy to Metafile*** or • Right-click in the chromatogram and choose ***Copy to Metafile*** *Result*: The ***Export Chromatogram to Windows Enhanced Metafile*** dialog opens. The only available file type is ***Enhanced Meta File*** (****.emf***)
2	• Select a destination folder. • Type a file name. • Click ***Save***.

Export Design of Experiments

Design of Experiments may be exported for use in the Design of Experiment application Modde. The design is exported in the specific format ***MODDE Investigation Project*** (****.mip***).

TIP: The evaluation of Design of Experiment results is described in the *UNICORN 6 Method Manual*.

Copy to the clipboard

You can use the Windows clipboard to copy the contents of the active window and paste it into other programs. The table below describes how to do this:

Step	Action
1	• Choose ***Edit:Copy*** or • Right-click and choose ***Copy to Clipboard*** or • Click the ***Copy*** icon *Result*: • If the active window only contains a chromatogram, it will immediately be copied to the clipboard as a Windows metafile. • If the active window also contains a peak table, the ***Copy to Clipboard*** dialog opens.
2	Select one of the options: • ***Copy curve window as enhanced meta file*** • ***Copy selected peak table data as tabbed text***
3	Click the ***OK*** button.

6 Other evaluation operations

Introduction

This chapter describes how the results can be used for other types of evaluations. It also contains information about how to automate frequently used evaluation procedures.

In this chapter

This chapter contains these sections.

Section	See page
6.1 Curve operations	207
6.2 Evaluate a column performance test	220
6.3 Automated evaluation procedures	224

6.1 Curve operations

Introduction

This section describes:

- How to evaluate single curve data points in chromatograms
- How to reduce the noice from curves and remove ghost peaks
- How to use arithmetic operations on curves

  and
- How to find slope values that can be used for example as watch instruction parameter values

In this section

This section contains these topics:

Section	See page
6.1.1 Evaluate single curve data points	208
6.1.2 Reduce noise and remove ghost peaks	212
6.1.3 Add, subtract or divide curves	214
6.1.4 Find slope values	217

6.1.1 Evaluate single curve data points

Introduction

It is possible to determine the coordinates of any point on a curve and to obtain values for retention and peak height. This is a useful tool for many other functions, such as for measuring the parameters used in baseline calculations.

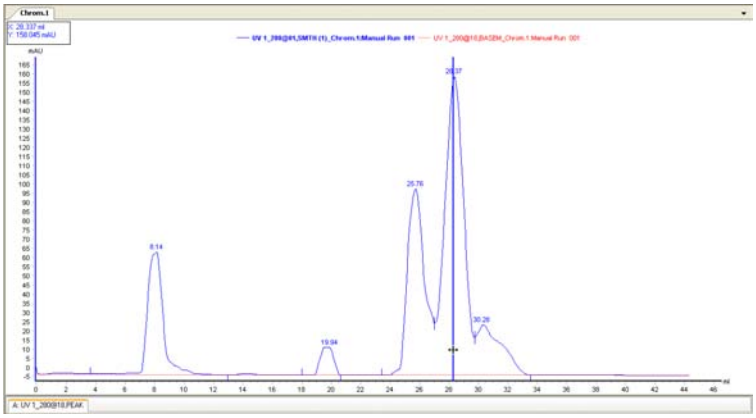
Measurement options

Coordinates can be obtained in two ways:

- Through direct measurement.
- From peak table data.

Direct measurements

The table below describes how to make direct measurements in a chromatogram:

Step	Action
1	Right-click in the chromatogram and select ***Vertical marker***. *Result*: A vertical line is set in the chromatogram. A text box in the top left corner of the chromatogram displays the X-axis and Y-axis values of the curve at the point where the marker line crosses the curve. See the illustration below:  **TIP:** The color of the ***Vertical marker*** is the same as the selected curve.
2	Move the marker with your mouse to display the peak position in x-axis and y-axis values. The mouse pointer changes into a double-headed arrow when it is properly placed over the marker.
3	Click the curve name legend above the chromatogram to change to another curve. *Result*: The Y-axis is changed to the one corresponding to the new curve.
4	Right-click and select ***Vertical marker*** again to de-select the function.

Set a reference point

The table below describes how to set a reference point:

Step	Action
1	• Right-click in the chromatogram and select ***Vertical marker***. • Move the marker with your mouse to the place for the reference point.
2	Right-click in the chromatogram and select ***Set vertical marker reference point*** to define a reference point for the marker position.
3	Drag the marker away from the reference point and read the X-axis and Y-axis values for the new position. You can also read the • ***Delta*** • ***Mean*** • ***Min*** and • ***Max*** curve values for the interval between the reference point and the new position.

Note: *The curve interval between the reference point and the new marker position is shaded in the chromatogram.*

Record a Snapshot

The table below describes how to record a ***Snapshot*** of the curve values at a specific volume or time point:

Step	Action
1	• Right-click in the chromatogram and select ***Vertical marker***. • Move the marker with your mouse to the place for the snapshot.
1	Right-click in the chromatogram and select ***Snapshot*** from the shortcut menu. *Result*: The ***Snapshot*** dialog opens.

Step	Action
2	The dialog displays all the curve data for the y-axis value where the snapshot was taken. • Click the ***Save*** button to save the snapshot as a text file. • Click the ***Print*** button to print the snapshot.
3	Click the ***Close*** button to close the dialog.

Select peak table data

The retention time and amplitude of any peak can be viewed directly in a peak table after an integration. These data and more are selected in the chromatogram ***Customize*** dialog. The table below describes how to select peak table data.

Step	Action
1	Click the ***Customize*** icon. *Result*: The ***Customize*** dialog opens.
2	Click the ***Peak Table*** tab of interest.
3	• Select the checkboxes on the ***Select peak table columns*** list for all items that you want to display in the table. • Click ***OK***.

6.1.2 Reduce noise and remove ghost peaks

Introduction

Sometimes the chromatograms contain curves with a noisy baseline. The noise can be caused by several factors, for example a dirty flow cell, air bubbles, electrical noise, dirty buffers, etc. The amount of noise can usually be reduced by taking proper precautions, for example filtration of buffers and instrument maintenance.

You can also use the smoothing function to reduce or remove background noise from a selected curve. Smoothing is always a compromise between noise removal and preservation of peak shape.

Smooth a curve

The table below describes how to select a smoothing function and smooth a curve:

Step	Action
1	Select ***Operations:Smooth***. *Result:* The ***Smooth*** dialog box is displayed.
2	Select the curve to be smoothed and its target destination.
3	Select the ***Filter type*** to be applied. The options are: • ***Moving average*** Use this if you have noise along most of the curve. It affects peak height but not retention. There is little effect on the peak area. • ***Autoregressive*** Use this if you have periodic noise along the whole curve. It affects peak height and retention, although this has little effect on the peak area. • ***Median*** Use this if there is only one or a few noise spikes, for example caused by air bubbles, or if the noise is confined to only a small part of the curve. It can flatten peaks and affect peaks areas slightly, but does not affect retention. • ***Savitzky-Golay*** Use this to calculate the smoothing and differentiation of data by a least squares technique.

Step	Action
4	Select an appropriate smoothing parameter value from ***Light*** to ***Hard*** for the selected filter in the ***Filter Parameters*** field. Use the slider, or insert a value manually in the text field. The manual value has the same unit as the chromatogram. The smoothing effect increases with increasing parameter values. • Click ***OK***. Result: A smooth curve is added to the chromatogram. By default, smoothed curves are given the suffix ***SMTH***. The default curve name can be changed as needed.

TIP: Start with a low parameter value, for example the default value, and increase it until the best result is achieved. A useful strategy is to increase the parameter value by the default value for each try.

6.1.3 Add, subtract or divide curves

Introduction

In some method runs, several sequential chromatograms might have been created. This can occur, for example, when the instruction ***New chromatogram*** has been used in the method, thus creating different chromatograms during the run. In order to view and evaluate the resultant curve of all the chromatogram parts, the curves must be added together. Usually, you have a number of chromatograms within the same result file and you want to add the curves. In some circumstances, curves might need to be imported from other result files.

Sometimes the presentation of a curve may benefit if a blank run curve or a baseline curve is subtracted from the curve. For example, this is useful when the curves have a drifting baseline or ghost peaks.

Curves may also be divided, for example to determine the ratio between curves as a means to study peak purity or to identify peaks. This is described in *Section 4.4.4 Peak purity and peak identification, on page 156*.

Add curves

The table below describes how to add several curves to form a combined, cumulative curve:

Step	Action
1	Select and view the first chromatogram in the sequence.
2	Choose ***Operations:Add***. *Result:* The ***Add*** dialog is displayed.
3	• Select the source chromatogram in the chromatogram list and the first curve in the desired sequence in the left ***Source chromatogram and curve*** field. • Select the chromatogram in the chromatogram list and the second curve in the sequence in the middle ***Source chromatogram and curve*** field. ***Note:*** *The suggested curve number is highlighted in the* ***Target chromatogram and curve*** *field. A default name is displayed in the* ***Curve name*** *box.* • Click ***OK*** to add the two curves together in a new result curve in the first source chromatogram.

Step	Action
4	• Open the ***Add*** dialog again to add more curves to the result curve. • Select the chromatogram in the chromatogram list and the result curve (***.ADD***) from the previous addition in the left field. • Select the chromatogram in the chromatogram list and the next curve in the sequence in the middle field. • Click ***OK*** to add the two curves together in a new result curve in the first source chromatogram.
5	Repeat step 4 until all curves have been added together. The final curve should be the cumulative curve for the whole run.

Note: *All curves created using the **Add** operation receive the **ADD** suffix by default. The default curve name can be changed as needed. The original curves are distinguished in the chromatogram by underlined curve names.*

Subtract curves

The table below describes how to subtract curves:

Step	Action
1	Choose ***Operations:Subtract***. *Result:* The ***Subtract*** dialog is displayed.
2	• Select the chromatogram in the chromatogram list and the first curve in the left ***Source chromatogram and curve*** field. • Select the chromatogram in the chromatogram list and the curve you want to subtract from the first curve in the middle ***Source chromatogram and curve*** field.
3	• Click the ***OK*** button. *Result*: The second curve is subtracted from the first and a new result curve of the difference is created in the first source chromatogram.

Note: *All curves created using the **Subtract** operation receive the **SUB** suffix by default. The default curve name can be changed as needed. The original curves are distinguished in the chromatogram by underlined curve names.*

Divide curves

The table below describes how to divide curves:

Step	Action
1	Choose ***Operations:Divide***. *Result:* The ***Divide*** dialog is displayed.
2	• Select the chromatogram in the chromatogram list and the first curve in the left ***Source chromatogram and curve*** field. • Select the chromatogram in the chromatogram list and the curve you want to divide the first curve with in the middle ***Source chromatogram and curve*** field.
3	If necessary, select the ***Threshold*** option and enter Y-axis threshold values. You may enter different values for each curve. This option is available only for the ***Divide*** operation. If one of the two curves that are divided has values below the threshold, the resulting curve value will be 1 at those data points.
4	• Click the ***OK*** button. *Result*: The first curve is divided by the second and a new result curve of the quotient is created in the first source chromatogram.

Note: *All curves created using the **Divide** operation receive the **DIV** suffix by default. The default curve name can be changed as needed. The original curves are distinguished in the chromatogram by underlined curve names.*

6.1.4 Find slope values

Introduction

With ÄKTAdesign systems it is only possible to collect peaks during fractionation. The way to find suitable slope values for a particular run is described in this section.

Where to use slope values

The slope values can be used in the ***Method Editor***

- as ***Start slope*** and ***End slope*** values in the ***Peak Fractionation Settings***.
- as parameters for the ***Watch*** instruction.

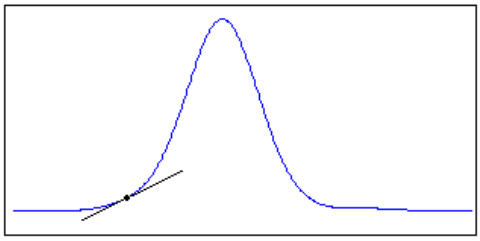
Using slope values for Watch instructions

Conditional ***Watch*** instructions can be set up to let the progress of a run be determined by the events during the run (e.g., start to collect fractions when the first peak emerges).

The slope of the curve can be set as a condition to satisfy a ***Watch*** condition in the method during the run. It is important to use accurate slope values for the specific ***Watch*** instruction parameter.

A sample run

You must first make a separation run with the sample you intend to purify. The result from this separation run is then used to find the slope values.



Retention scale

Time should be used as the X-axis scale for retention.

Step	Action
1	Click the ***Customize*** icon. *Result*: The ***Customize*** dialog opens.
2	• Click the ***X-axis*** tab. • Select ***Time*** in the ***Base*** field. • Click ***OK***.

Differentiate the curve

The slope values are measured on a differentiated curve. The table below describes how to create a differentiated curve.

Step	Action
1	Select ***Operations:Differentiate***. *Result*: The ***Differentiate*** dialog opens.
2	• Select the chromatogram and the UV curve you want in the ***Source chromatogram and curve*** list. • Click the ***First order*** option button.
3	• Click ***OK***. *Result*: The differentiated curve opens in the chromatogram.

Measure the slope values

Sometimes the differentiated curve must be filtered to reduce noise and ghost peaks before the measurements. See section *Section 4.4.4 Peak purity and peak identification, on page 156*.

The table below describes how to measure the slope values on the differentiated curve.

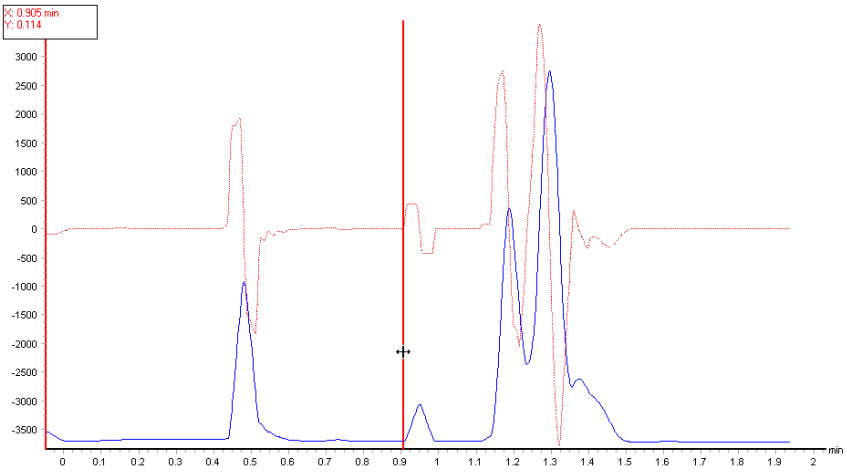
Step	Action
1	Select the differentiated curve (The text ***Differentiation*** will be shown in brackets above the Y-axis.).
2	Use the zoom function to magnify the curve over an appropriate area.
3	Right-click and select ***Vertical marker*** from the shortcut menu. *Result*: A vertical marker bar opens in the chromatogram.
4	Place the marker at the beginning of a peak where you want the ***Watch*** conditions to be fulfilled (i.e. where the slope becomes higher).
5	Read the actual slope value in the active marker text box in the top left corner of the chromatogram pane.

Note: *The unit for the differentiated curve is mAU/min or AU/min. Any Y-axis value for the differentiated curve is the UV curve slope at the selected retention point.*

TIP: Measure the slope at the beginning and the end of the smallest, flattest peak of all the peaks of interest, and use these values.

Illustration: Slope value measurement

The illustration below shows a measurement of the slope limit after differentiation:



6.2 Evaluate a column performance test

Introduction

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). The column performance test calculates these values, which can be stored as statistics in the Column Logbook.

Note: *The calculated plate number will vary according to the test conditions and it should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.*

Theory of HETP and As

The column performance test calculates HETP and A_s from the UV curve (or conductivity curve).

HETP

$$HETP = \frac{L}{N}$$

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

where:

L = Bed height (cm)

N = number of theoretical plates

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units.

Peak asymmetry factor

$$A_s = \frac{b}{a}$$

where:

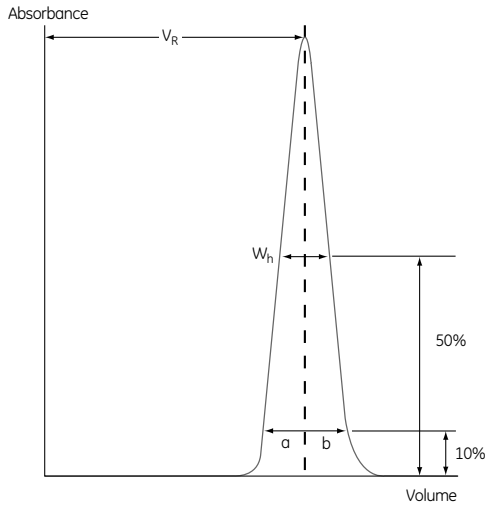
a = 1^{st} half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height.

The peak should be symmetrical, and the **Asymmetry factor** as close to 1 as possible. Values between 0.8–1.5 are usually acceptable. A change in the shape of the peak is usually the first indication of bed deterioration due to use.

Illustration of a curve

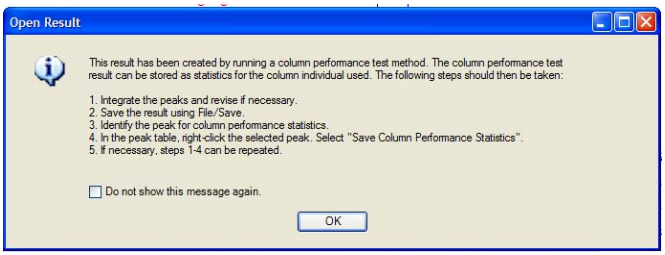
The illustration below shows a UV curve for acetone in a typical test chromatogram in which the HETP and A_s values are calculated.



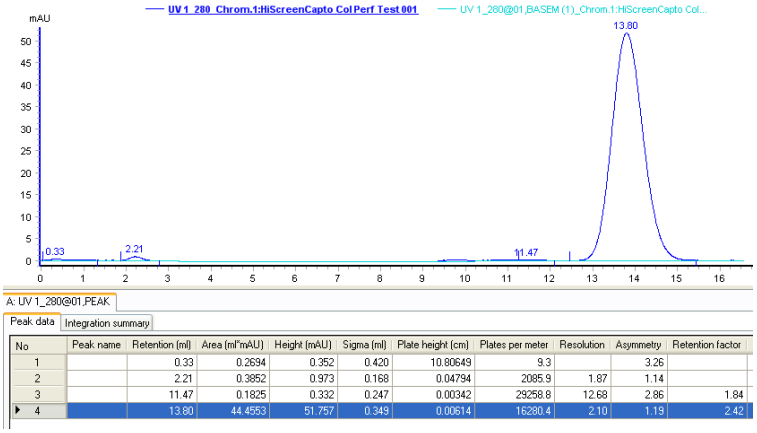
Evaluate the HETP test

Follow the instruction below to evaluate a column performance test.

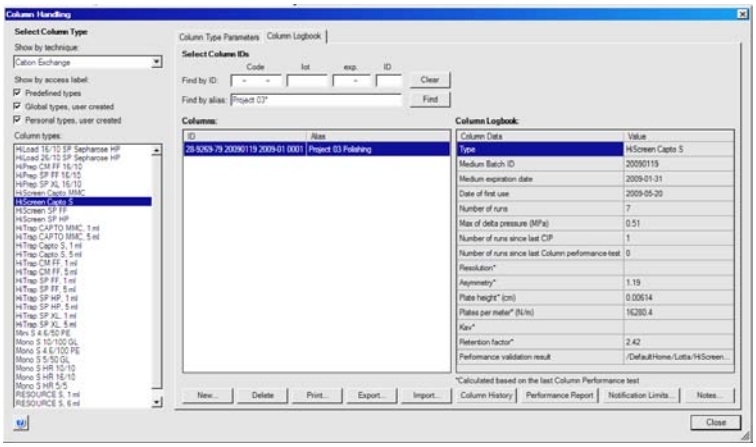
Step	Action
1	Browse for and open the column performance test result in the ***Result Navigator*** in the ***Evaluation*** module. *Result*: The ***Open Result*** dialog is displayed and describes how to save the statistics in the ***Column Logbook***.


Open Result
This result has been created by running a column performance test method. The column performance test result can be stored as statistics for the column individual used. The following steps should then be taken:
1. Integrate the peaks and revise if necessary.
2. Save the result using File/Save.
3. Identify the peak for column performance statistics.
4. In the peak table, right-click the selected peak. Select "Save Column Performance Statistics".
5. If necessary, steps 1-4 can be repeated.
Do not show this message again.
OK

2	Perform a peak integration, see *Section 4.2.2 Perform a peak integration, on page 101* steps 1-3. Select the UV (280) curve.
3	Select the peak for column performance statistics in the peak table by clicking the peak number ***No***.


UV 1_280_Chrom.1:HiScreenCapto Col Perf Test 001
UV 1_280@01,BASEM (1)_Chrom.1:HiScreenCapto Col...
mAU
13.80
0.33
2.21
11.47
A: UV 1_280@01,PEAK
Peak data
Integration summary
No | Peak name | Retention (ml) | Area (ml*mAU) | Height (mAU) | Sigma (ml) | Plate height (cm) | Plates per meter | Resolution | Asymmetry | Retention factor
1 | 0.33 | 0.2694 | 0.352 | 0.420 | 10.80649 | 9.3 | | 3.26 |
2 | 2.21 | 0.3852 | 0.973 | 0.168 | 0.04794 | 2085.9 | 1.87 | 1.14 |
3 | 11.47 | 0.1825 | 0.332 | 0.247 | 0.00342 | 29258.8 | 12.68 | 2.86 | 1.84
4 | 13.80 | 44.4553 | 51.757 | 0.349 | 0.00614 | 16280.4 | 2.10 | 1.19 | 2.42

4	To store the column performance result as statistics in the Column Logbook, right-click the selected row and select ***Save Column Performance Statistics***.

Step	Action
5	• Click the ***Column Handling*** icon. • Select the ***Column Logbook*** tab. *Result*: Column Performance statistics is displayed in the ***Column Logbook*** table. The ***Column Data*** include ***Asymmetry***, ***Plate height*** and **Plates per meter**. 

6.3 Automated evaluation procedures

Introduction

Frequently used evaluation procedures can be recorded and saved. The saved sequence of procedure instructions can be included as part of a method. When the method is run, the evaluation procedures will be performed automatically. This section decribes how to create and edit procedures and how to work with the ***Procedure Editor***.

In this section

This section contains these sub-sections.

Section	See page
6.3.1 Create a new procedure	225
6.3.2 Edit a procedure	228
6.3.3 Run a procedure	231
6.3.4 Rename or remove procedures	235

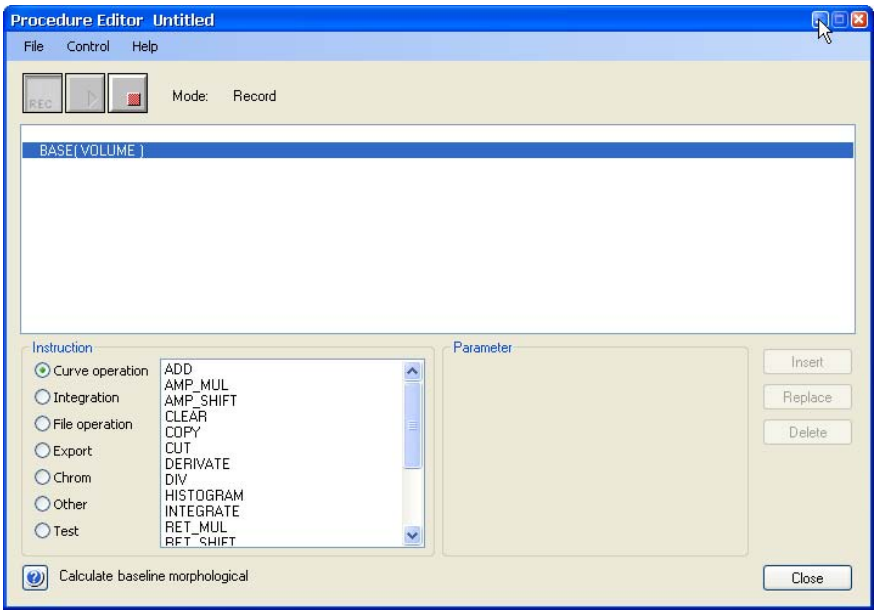
6.3.1 Create a new procedure

Introduction

You can use the ***Procedure Editor*** to record or create a new procedure. The ***Procedure Editor*** can also be used to view and edit the instructions within a procedure. This section describes how to use the ***Procedure Editor*** to record new procedures.

The Procedure Editor dialog

The illustration below shows the ***Procedure Editor*** in ***Record mode***.



Record a procedure

The table below describes how to record a new procedure.

Step	Action
1	Open a result file in the ***Evaluation*** module.

Step	Action
2	Choose ***Procedures:New***. *Result*: The ***Procedure Editor*** dialog opens in ***Edit*** mode.
3	**Start the recording** • Choose ***Control:Record*** or • click the ***REC*** icon.
4	Minimize the ***Procedure Editor*** dialog.
5	Perform the evaluation steps that the procedure is to contain. *Result*: The steps are recorded in the order that they are performed.
6	**Stop the recording** • Restore the minimized ***Procedure Editor*** dialog box and select ***Control:End Record***. or • Restore the minimized ***Procedure Editor*** dialog and click the ***Stop*** button.
7	Choose ***File:Save*** or ***File:Save As*** in the dialog. *Result*: The ***Save As*** dialog opens.
8	• Type a name for the new procedure in the ***Procedure name*** text field. • Select the ***Global procedure*** checkbox if desired (see further information below). • Click ***OK***. *Result*: The procedure is saved and available for future use, for example as part of a method.
9	Click the ***Close*** button to close the dialog.

Create a Global procedure

You can choose to save the new procedure as a ***Global procedure***. This makes the procedure available to all users. The procedure will have ***(Global)*** before the name to designate that it is available to all users.

Note: *You must belong to an* ***Access Group*** *with* ***Create/Edit Global Objects*** *authorization to be able to save* ***Global procedures***.

Build a procedure with instructions

You can select instructions in the ***Procedure Editor*** dialog to build a complete procedure step by step. The procedure instructions are described in *Section A.4 Evaluation procedure instructions, on page 263*. The table below describes how to create a new procedure with instructions.

Step	Action
1	Choose ***Procedures:New***. *Result*: The ***Procedure Editor*** opens in ***Edit*** mode.
2	• Select an instruction type from the ***Instruction*** list (e.g., ***Curve operation***). • Select the appropriate instruction from the field to the right of the instruction types. • Select or type the appropriate parameter values in the ***Parameter*** field. • Click ***Insert***.
3	Repeat step 2 until the procedure is complete.
4	Choose ***File:Save***.
5	Type a procedure name and click ***OK***.
6	Click the ***Close*** button in the ***Procedure Editor***.

6.3.2 Edit a procedure

Introduction

Evaluation operations are represented by instructions in the ***Procedure Editor*** dialog box. The instructions can be modified to suit other specific evaluation needs and be saved for later use. This section describes how to use the ***Procedure Editor*** to edit a procedure.

Edit a procedure

The table below describes how to edit an existing procedure:

Step	Action
1	Select ***Procedures:Edit:Open***. *Result*: The ***Open Procedure*** dialog opens.
2	Select the procedure from the list and click ***OK***. *Result*: The ***Procedure Editor*** opens in ***Edit*** mode.
3	Select an instruction in the procedure window. *Result*: The instruction and the corresponding parameters are displayed in the ***Instruction*** and ***Parameter*** fields. A short definition of the selected instruction is displayed at the bottom left corner.
4	Type or select new values in the ***Parameter*** fields and click the ***Replace*** button. *Result*: The old parameters are replaced by the new parameters.
5	**Add a new instruction** • Select the instruction in the procedure immediately before where you want the new instruction. • Select a type and an instruction in the ***Instruction*** field. • Select or type parameter values in the ***Parameter*** field. • Click the ***Insert*** button. *Result*: The new instruction is inserted after the selected instruction.
6	**Remove an instruction** Select an instruction in the procedure and click the ***Delete*** button to remove the instruction from the procedure.

Step	Action
7	• Choose ***File:Save*** and • click the ***Close*** button to close the dialog.

Descriptions of the procedure instructions

Appendix *Section A.4 Evaluation procedure instructions, on page 263* contains a list of procedure instructions with descriptions.

Add instructions to a procedure by recording

If you start recording again you can add more instructions to a procedure that is already open in the ***Procedure Editor***:

- The new instructions will be added to the end of the present procedure.

  or

- The new instructions will be inserted after the selected instruction if an instruction has been selected.

Invalid instructions

The procedure will stop and display an error message if an instruction calls for an invalid operation when the procedure is run. Any subsequent instructions in the procedure will not be executed.

Address the right curves

Curves are identified only by their storage position. An instruction can become invalid if it addresses the wrong curve:

Example

- The instruction ***ADD (01,02,03)*** will try to add curve 01 to curve 02 and store the result in position 03.

- A curve in position 03 that is not a raw data curve will be overwritten.
- A raw data curve in position 03 cannot be overwritten and the procedure will be stopped at that point.

Default values for classic baseline instructions

When a classic or morphological algorithm is used to calculate a baseline, UNICORN will suggest default values for the four control parameters based on the appearance of the curve. To instruct UNICORN to use default values appropriate for the curve every time the procedure is run, choose the default setting in the appropriate fields for the parameters.

Example

- `CALCULATE_BASELINE (01, 06, XXX, XXX, XXX, XXX)`

  Can be changed to:

- `CALCULATE_BASELINE (01, 06, DEFAULT, DEFAULT, DEFAULT, DEFAULT)`

Global procedures

It is not advisable to edit existing global procedures. Open the global procedure instead and save a copy under a new name. Use this copy for editing purposes.

6.3.3 Run a procedure

Introduction

You can run the saved procedures either for a specific chromatogram or for multiple chromatograms.

Run a single procedure

The table below describes how to run a procedure for a single, specific chromatogram.

Step	Action
1	Open a result file.
2	Select ***Procedures:Run***. *Result*: The ***Open Procedure*** dialog opens.
3	Select the procedure from the list and click ***OK***. *Result*: The procedure is executed. A confirmation message is displayed.

TIP: You can also open the procedure in the ***Procedure Editor*** dialog and choose ***Control:Run*** or click the ***Play*** icon.

Run on multiple chromatograms

It is possible to apply an evaluation procedure to a designated batch of several result files if they are not open in the ***Evaluation*** module. An open file will not run and an error message will be displayed.

The run on multiple chromatograms is performed in the background of the ***Evaluation*** module and the results of the run are not seen, with the exception of prints and documentation that are defined as steps in the procedure. For example, runs on multiple chromatograms are useful

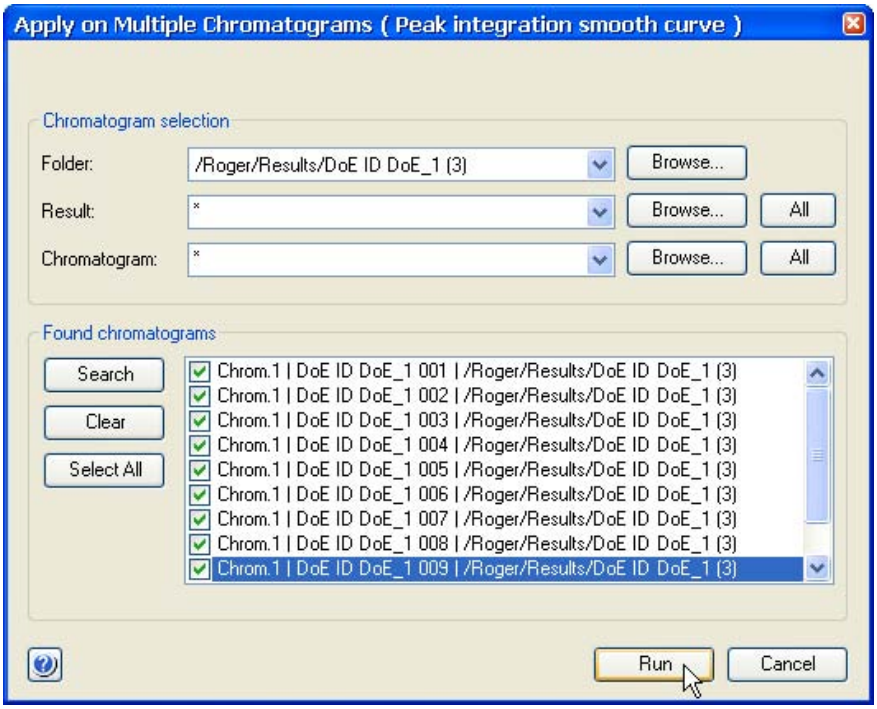
- to perform integration with the same parameter settings on many results,

  or

- to print a number of results with the same settings.

The Apply on Multiple Chromatograms dialog

The illustration below is an example of search results in the ***Apply on Multiple Chromatograms*** dialog:


Apply on Multiple Chromatograms (Peak integration smooth curve)
Chromatogram selection
Folder:
/Roger/Results/DoE ID DoE_1 (3)
Browse...
Result:
*
Browse...
All
Chromatogram:
*
Browse...
All
Found chromatograms
Search
Clear
Select All
Chrom.1 | DoE ID DoE_1 001 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 002 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 003 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 004 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 005 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 006 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 007 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 008 | /Roger/Results/DoE ID DoE_1 (3)
Chrom.1 | DoE ID DoE_1 009 | /Roger/Results/DoE ID DoE_1 (3)
Run
Cancel

Perform a procedure run on multiple chromatograms

The table below describes how to perform a batch run on multiple chromatograms:

Step	Action
1	Choose ***Procedures: Run on Multiple Chromatograms***. *Result*: The ***Open Procedure*** dialog opens.
2	Select the procedure from the list and click ***OK***. *Result*: The ***Apply on Multiple Chromatograms*** dialog opens.

Step	Action
3	Use the ***Browse*** buttons to find and select the folders, result files and chromatograms you want to search. Click the mouse button while pressing ctrl to select several items. **TIP:** The search will only be performed in the selected folder, result and/or chromatogram. You can use standard wildcard characters and define restricting search criteria for the ***Result*** and ***Chromatogram*** fields. Up to 10 user-defined search filters can be saved in the drop-menus.
4	Click the ***Search*** button. *Result*: A list of found chromatograms is displayed.
5	Select the chromatograms you want to perform the run on. • The ***Select All*** button selects all chromatograms. • The ***Clear*** button removes all chromatograms from the list.
6	Click the ***Run*** button. *Result*: The evaluation procedure is performed on each of the selected chromatograms and any created curve or peak table will automatically be saved in each result file. ***Note:*** *A **Batch Run Log** will open after the last procedure run is performed, listing any chromatogram where the procedure was unable to run. This dialog does not open if all runs are successful.*

Generate reports from multiple chromatograms

Evaluation procedures applied on multiple chromatograms can be a useful tool to produce printed documentation simultaneously for many result files (e.g., for a number of scouting runs). The table below describes how to create a procedure to generate reports for several results.

Step	Action
1	Start the recording of a new procedure and minimize the ***Procedure Editor***.
2	Choose ***File:Report***. *Result*: The ***Generate Report*** dialog opens.

Step	Action
3	Choose a report format.
4	• Click the ***Print*** button. *Result*: The ***Print*** dialog opens. • Click the ***OK*** button. • Click the ***Close*** button to close the ***Generate Report*** dialog.
5	Maximize the ***Procedure Editor*** and click the stop button.
6	Save the procedure. **TIP:** A printing procedure can also be saved with a method, to produce automatic prints at the end of a run.

Note: *When a procedure is applied on several results, the latest version of the procedure will be used. However, procedures that are saved with a method are not affected if the original procedure is edited at a later time.*

Add procedures to the Quick Menu

You can add selected evaluation procedures to the ***Procedures*** menu in the ***Evaluation*** module. The table below describes how to add procedures to the menu:

Step	Action
1	Select ***Procedures:Edit Quick Menu***. *Result*: The ***Edit Procedures Menu*** dialog opens.
2	• Select the checkboxes of the procedures you want to display on the menu. • Click ***OK***. *Result*: The selected procedures are included on the ***Procedures*** menu.

Remove a procedure

Open the ***Edit Procedures Menu*** dialog and select the checkbox again to de-select and remove a procedure from the menu.

6.3.4 Rename or remove procedures

Introduction

The procedures that you have created can be renamed or removed from the list of available procedures. This section describes how this is done.

Rename a procedure

The table below describes how to rename a procedure.

Step	Action
1	Choose ***Procedures:Edit:Rename***. *Result*: The ***Rename Procedure*** dialog opens.
2	Select a procedure. *Result*: The procedure name is displayed in the ***New Name*** text box.
3	Type the new name.
4	Click ***OK***. *Result*: The procedure name is changed.

Delete a procedure

The table below describes how to delete a procedure.

Step	Action
1	Choose ***Procedures:Edit:Delete***. *Result*: The ***Delete Procedure(s)*** dialog opens.
2	Select a procedure.
3	• Click ***OK***. • Click the ***Yes*** button to confirm. *Result*: The procedure is deleted.

Global procedures

It is not advisable to edit existing global procedures. Open the global procedure instead and save a copy under a new name. Use this copy for editing purposes.

7 Troubleshooting

Introduction

This appendix describes different problems that may arise when performing result evaluations in UNICORN, and how to solve the problems. It also decribes how to generate a system error report describing performance problems.

In this chapter

This appendix contains these sections:

Section	See page
7.1 Troubleshooting: Evaluation	238
7.2 System Error Reports	241

7.1 Troubleshooting: Evaluation

In this section

This section describes how to solve the following evaluation problems:

- Evaluation procedures abort
- Maximum number of curves exceeded
- Distorted peaks
- Column performance test errors
- Retention time differences
- Import UNICORN 5 result errors
- Result not found

Evaluation procedure aborts

The table below describes a problem and its solution:

Problem description	Solution
The evaluation procedure aborts	Instructions in an evaluation procedure refer to curves by identification number irrespective of the curve names. Make sure that the curves processed when the procedure is executed are compatible with those processed when the procedure was recorded. An evaluation procedure aborts if you try to store the resulting curves at the position of an original raw data curve.

Maximum number of curves exceeded

The table below describes a problem and its solution:

Problem description	Solution
The maximum number of curves (100) are exceeded when importing curves	The injection curve is also included for each curve that is imported. Delete the unnecessary curves before importing more curves.

Distorted peaks

The table below describes a problem and its solution:

Problem description	Solution
Peaks appear distorted	A high averaging time value will distort and delay the peaks. A high UV averaging time shall only be used when very large and broad peaks are expected.

Column performance test errors

The table below describes problems and their solutions:

Problem description	Solution
The Column Performance Test attribute is missing in the ***Run History***	Verify that the proper steps have been performed during the evaluation.
The column performance parameter values are wrong	Verify that the adjust zero retention value is set to the injection.

Retention time differences

The retention value in the peak label in the chromatogram may differ from that in the peak table or that in the x-axis. The table below describes problems and their solutions:

Problem description	Solution
The retention time in the peak table differs from that in the chromatogram.	Verify that the integration was performed on the currently displayed chromatogram. ***Note:*** *Only applicable if there are more than one chromatogram in the result.*
The retention value in the peak label differs from that in the x-axis.	Verify that the x-axis base has not changed after the peak integration was performed, but is the same as in the peak table. This is applicable for ml and CV bases.

Problem description	Solution
The retention value is not displayed in the peak label.	• Verify that the x-axis base has not changed after the peak integration was performed, but is the same as in the peak table. This is applicable for volume and time bases. • Verify that the chromatogram has not been changed, for example by selecting or deselecting ***Adjust retention zero to injection number*** in the ***Customize*** dialog, ***X-Axis*** tab.

Import UNICORN 5 result errors

The table below describes a problem and its solution:

Problem description	Solution
Not possible to import UNICORN 5 results.	• Verify that none of the suggested actions in the dialog are applicable. - A result with the same name already exists. - You do not have sufficient access rights. - The database is inaccessible. • Make sure that the result is created by UNICORN 5 control software and that the system is supported for data migration to UNICORN 6, i.e., ÄKTAexplorer or ÄKTApurifier, see UNICORN 6 Administration and Technical Manual. • Verify that the result can be opened in UNICORN 5, to make sure that the file is not corrupt. • Verify that the column that was used had ml as column volume unit.

7.2 System Error Reports

Introduction

The ***Generate System Error Report Wizard*** is used to generate problem reports. The report can provide useful background information for the support staff when trying to provide solutions to problems or suggestions for improved system performance.

Step 1: Create an error report

This table below describes how to activate the ***Generate System Error Report Wizard*** and create a report:

Step	Action
1	• Choose the ***Reports:Create System Error Report*** menu command in the ***Administration*** module [1]. *Result*: The ***Generate System Error Report Wizard*** opens. ***Note:*** *If an error occurs during a method run you can also start the wizard by clicking the* ***Report*** *button in the error message dialog.* • Click the ***Next*** button.

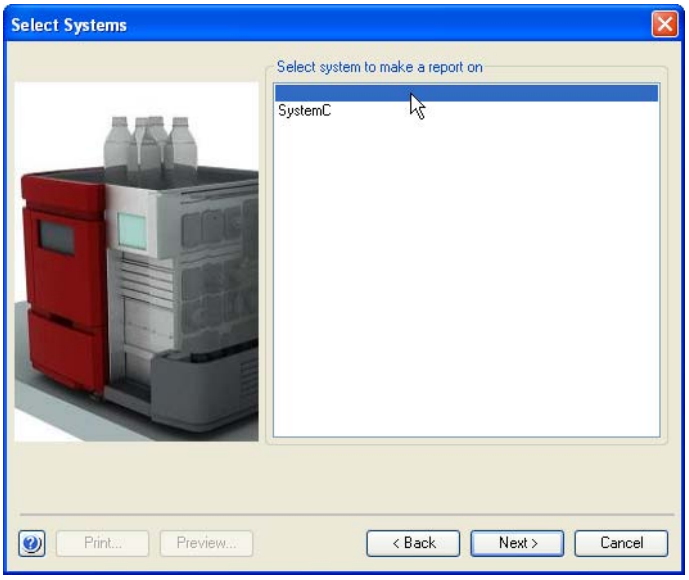
1 You can also create an error report from ***System Control*** by choosing ***System:Create System Error Report***. A system must be connected. The report will be created for the connected system and step 2 in the instruction above will be omitted.

Step	Action
2	The ***Select Systems*** dialog is displayed, showing all accessible systems.

TIP: In this and all subsequent dialogs you can always click the ***Back*** button to return to previous dialogs and change the entries.

- Select the system that the error is connected to.
- Click the ***Next*** button.

Note: *If the problem is general, related to the UNICORN software and cannot be connected to a specific system you do not have to select a system in this step. Ensure that the empty space above the first system is selected (as illustrated below) and click* ***Next*** *to proceed. However, it is an advantage in subsequent troubleshooting if at least one system can be referenced.*



Step	Action
3	The ***Error Description*** dialog opens.

Add the following information:

- A short description of the problem.
- The circumstances under which the problem occurs.
- The consequences of the problem.

Click the ***Next*** button.

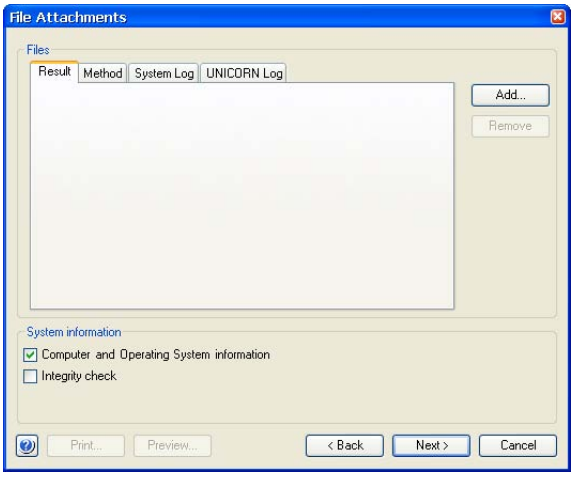
Step	Action
4	The ***Error Reproducibility*** dialog opens. • Specify whether the problem is reproducible or not. Select one of these alternatives: • - ***Yes*** (Provide a short description in the text box of how the problem can be reproduced.) - ***No*** - ***Unknown***. • Click the ***Next*** button. *Result*: The ***File Attachment*** dialog opens.
5	Go to step 2 below.

Step 2: Attach example files

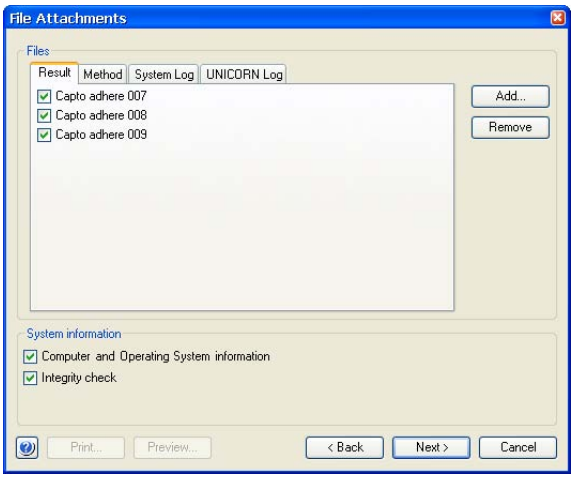
You can attach results, methods and/or UNICORN log files to the problem report.

The table below describes how to attach a file:

Step	Action
1	The ***File Attachments*** dialog box is displayed:



Step	Action
2	• Depending on the character of the file to be attached, select the appropriate tab: ***Result***, ***Method***, ***System Log*** or ***UNICORN Log***. • Attach a file: - Click the ***Add*** button. - Select a file in the dialog and click the ***Attach*** button. *Result*: The selected file is added to the tab in the ***File Attachments*** dialog.



TIP: To remove a file from the tab, select the checkbox and click the ***Remove*** button.

When attaching the ***UNICORN Log***, a separate dialog will open. Choose the time period for the attached log.

Step	Action
3	To include more information in the report, select the appropriate check boxes in the ***System information*** field. **Computer and Operating System information** A summary of the computer and operating system information, for example type of processor, processor speed, RAM, hard disk capacity and printer. **Integrity check** When UNICORN is installed a checksum calculation is performed on the stationary files (*.dll and *.exe) for the system. An integrity check means that a new checksum calculation is performed for the same files in their folders. This new calculated value is compared to the checksum value obtained during installation. The results of the comparison are presented in the report and any deviations are included. • Click the ***Next*** button. *Result*: The ***Generate System Error Report*** dialog is displayed.
4	Go to step 3 below.

Step 3: Generate and save the report

The table below describes how to generate and save the report.

Step	Action
1	By default, the report is saved as a zip file in the UNICORN folder on your local computer. If you want to save the report in another location, click the ***Browse*** button and select a destination folder.
2	You also have these options: • Click the ***Preview*** button to open the report in Notepad. • Click the ***Print*** button to print the report without any preview.
3	• Click the ***Finish*** button. *Result*: The report is generated and saved and the wizard dialog closes.

Appendix A Evaluation functions and instructions

Introduction

This appendix contains background information about the algorithms and calculation theories applied in UNICORN.

It also describes the peak table column components and the instructions that are available for automated evaluation procedures.

In this appendix

This appendix contains these sections:

Section	See page
A.1 Smoothing algorithms	247
A.2 Baseline calculation theory	250
A.3 Peak table column components	256
A.4 Evaluation procedure instructions	263

A.1 Smoothing algorithms

Introduction

This section describes how the smoothing functions are calculated. Choose ***Operations:Smooth*** in the ***Evaluation*** module to view and edit the options. Use of the ***Smooth*** dialog is described in *Section 6.1.2 Reduce noise and remove ghost peaks, on page 212*.

Moving average

The table below describes the process when the ***Moving average*** smoothing algorithm is used.

Stage	Description
1	For each data point in the source curve, the processed curve is calculated as the average of the data points within a window centered on the source data point. • The width of the window is determined by the ***Filter Parameter*** value, expressed as number of data points.
2	When the source point is less than half the window size from the beginning of the end of the curve, the average is calculated symmetrically round the source point over as many data points as possible. • If you increase the window width, the smoothing effect is also increased.

Note: *The filter algorithm only accepts odd integer parameter values between 1 and 151. If an even number has been given, it is incremented by adding the number one (1).*

Autoregressive

The table below describes the process when the ***Autoregressive*** smoothing algorithm is used:

Stage	Description
1	The first data point in the source curve is copied to the processed curve.
2	For each subsequent data point, the previous processed point is multiplied with the filter parameter value and added to the current source data point.

Stage	Description
3	The result is then divided by the parameter value plus 1 according to the following formulae: $t_1 = S_1$ $t_n = \frac{(p * t_{n-1} + S_n)}{(p+1)}$ Where: t_n = current processed point t_{n-1} = previous processed point S_n = current source point p = smoothing parameter value ***Note:*** *If you increase the parameter value, the smoothing effect is also increased.*

Note: *The filter algorithm only accepts integer parameter values between 1 and 25.*

Median

The table below describes the process when the ***Median*** smoothing algorithm is used.

Stage	Description
1	For each data point in the source curve, the processed curve is calculated as the median of the data points within a window centered on the source data point. • The width of the window is determined by the parameter value, expressed as number of data points.
2	When the source point is less than half the window size from the beginning of the end of the curve, the median is calculated symmetrically round the source point over as many data points as possible. • If you increase the window width, the smoothing effect is also increased. • To completely remove a noise spike, the window width should in effect be slightly more than twice the width of the spike.

Note: *The filter algorithm only accepts odd integer parameter values between 1 and 151. If an even number has been given, it is incremented by adding the number one (1).*

Savitzky-Golay

The table below describes the process when the ***Savitzky-Golay*** smoothing algorithm is used.

Stage	Description
1	The algorithm is based on performing a least squares linear regression fit of a polynominal of degree k over at least k+1 data points around each point in the curve to smooth the data. The derivative is the derivative of the fitted polynominal at each point. The calculation uses a convolution formalism to calculate 1st through 9th derivatives.
2	The calculation is performed with the data in low X to high X order. If the input trace goes from low to high, it is reversed for the calculation and is re-reversed afterwards.

TIP: See Gorry, Peter A, *General Least-Squares Smoothing and Differentation by the Convolution (Savitsky-Golay) Method* (Analytical Chemistry 1990, Volume 62, 570-573) for information about the Savitzky-Golay algorithm.

A.2 Baseline calculation theory

Introduction

There are two methods to calculate the baseline, based on a morphological and classic algorithm.

Overall process

The table below describes the overall process of a baseline calculation.

Stage	Description
1	The baseline segments are defined.
2	The baseline points are selected.
3	The baseline is drawn.

Baseline segment definition

Baseline parameters are used to find the baseline segments. The default values for the parameters are determined from the source curve. The baseline segments are found by different parameters that are based on the type of algorithm that is selected.

TIP: The parameters can be displayed in the ***Evaluation*** module if you choose ***Integrate:Calculate baseline*** function. You can also click the ***Baseline settings*** button in the ***Integrate:Peak integrate*** dialog.

Morphological algorithm

The ***Morphological*** algorithm searches for all parts of the source curve where:

- The curve parts come into contact at both ends of a horizontal line of the length defined in the ***Structure width*** parameter. The default value of this parameter is based on the widest detected peak in the curve. The horizontal line is moved along the curve up the peak until it reaches the contact points. The curve parts below the horizontal line and the line will now form a "curve" with a plateau. The center point in the plateau formed by the horizontal line will be the data point for the baseline.
- The data points fulfil the ***Minimum distance between data points***. This parameter reduces the total number of data points that are created from a curve.

Classic algorithm

The ***Classic*** algorithm searches for all parts of the source curve where:

- The curve parts are longer than the ***Shortest baseline segment***. This parameter determines the minimum length for a part of the source curve to be considered a possible baseline segment.
- The curve has no point outside the ***Noise window***. The noise window is defined as a rectangular corridor parallel to the slope of the curve and centered on the first and last points within the currently inspected segment.
- The slope is less than the ***Slope limit***. This limits the maximum slope of the baseline to differentiate baseline segments from peaks.
- The curve parts are lower than the ***Max baseline level***. This parameter determines the highest acceptable signal level for the baseline.

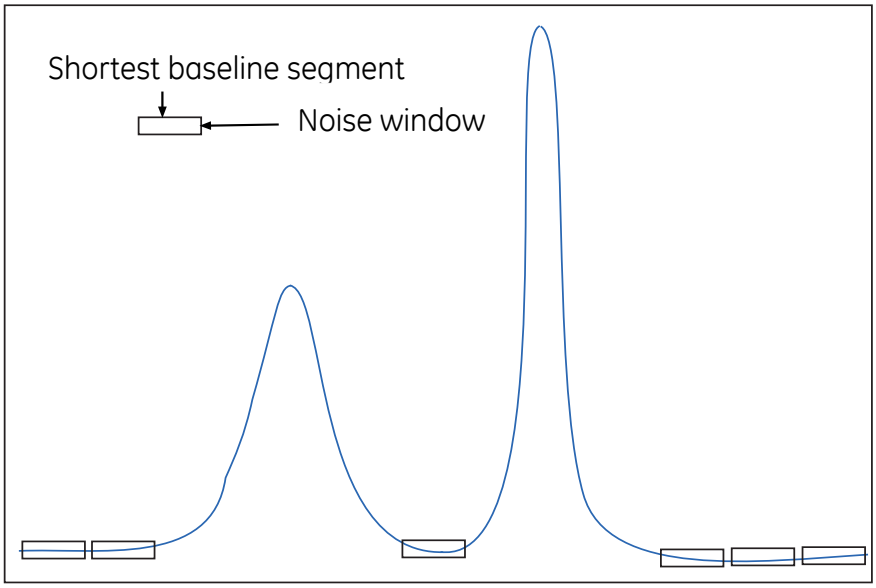
Baseline parameters

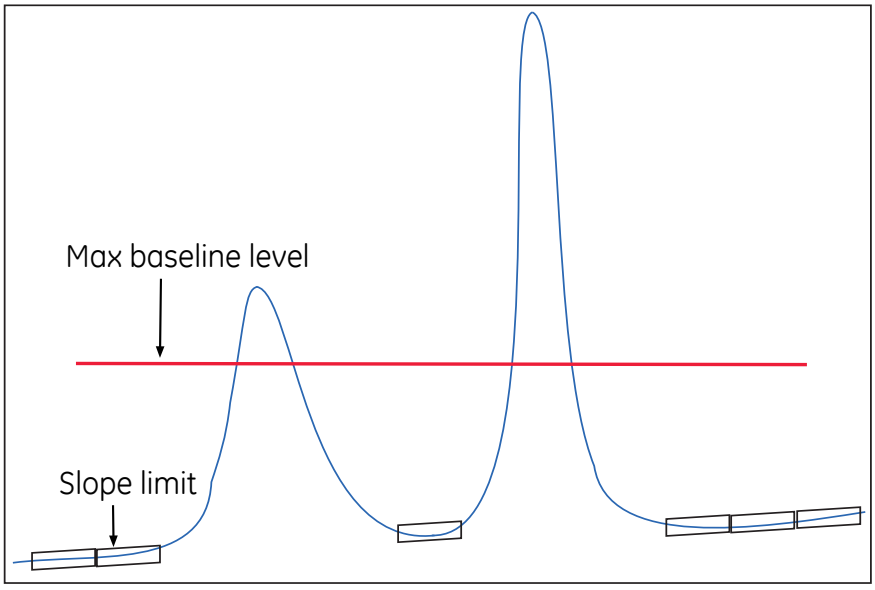
The baseline parameters can be illustrated as a rectangular box that the source curve has to fit into in order to be identified as a baseline segment, where:

- The length of the box corresponds to the ***Shortest baseline segment***.
- The height of the box corresponds to the maximum level of noise on the baseline segments. This is referred to as the ***Noise window***.
- The box is allowed to be tilted with a maximum slope corresponding to the ***Slope limit***.
- The box is not allowed to move up above the ***Max baseline level***.

Baseline parameters - illustration

The illustrations below show the baseline parameters graphically.





Baseline segment identification

The table below describes the baseline segment identification process:

Stage	Description
1	The box is virtually moved along the source curve in steps of one third of the ***Shortest baseline segment*** length to look for baseline segments.
2	A baseline segment is found whenever the currently examined part of the source curve fits completely within the box.
3	The found baseline segments are joined by connecting adjacent segments, provided that the slope of the joining lines does not exceed the ***Slope limit***.

Baseline points (Classic algorithm)

When the baseline segments have been defined and joined, they are replaced by baseline points at the start and end of each segment. The line between these is also filled with points.

TIP: The baseline points are shown as gray squares in the ***Integrate:Edit baseline*** function of the ***Evaluation*** module.

Baseline drawing (Classic algorithm)

The baseline points are used to create the baseline curve using a spline interpolation. The spline function ensures that the baseline curve is guided by the baseline points. However, the curve does not necessarily pass through the baseline points. The baseline will be a smoothly curved function passing close to or through the points.

To reduce the effect of noise at the peak integration, the created baseline is forced equal to the source curve in every position where the difference between the baseline and the source curve is small enough. Choose ***Integrate:Calculate Baseline***. If the ***Accept negative peaks*** option is off, the baseline will be forced down to the level of the source curve whenever the created baseline goes above the source curve.

Measure a Classic algorithm baseline segment

You can try to measure the ***Shortest baseline segment*** length directly on your chromatogram. The table below describes how to do this:

Step	Action
1	Locate the shortest segment of the curve that you consider a part of the baseline.
2	Right-click in the chromatogram and choose ***Vertical marker***.
3	Use the ***Vertical marker*** to measure the length of the segment by reading the start and end point values.
4	Choose ***Integrate:Calculate Baseline*** and insert this value as the ***Shortest baseline segment*** value.

Measure noise level (Classic algorithm)

Curve coordinates can also be used to measure noise levels on the source curve. The table below describes how to do this:

Step	Action
1	Use the ***Zoom*** function to focus on a part of the curve that is representative for the baseline noise.
2	Select an appropriate Y-axis scale.
3	Measure the Y-axis coordinates.
4	• Calculate the noise range as the difference between the max. and min. values. • Add an extra 20%. • Choose ***Integrate:Calculate Baseline*** and insert this value as the ***Noise window*** value.

Measure the slope limit (Classic algorithm)

The table below describes how to measure the slope at any part of the curve.

TIP: This is also described in *Section 6.1.4 Find slope values, on page 217*.

Stage	Description
1	Select ***Operations:Differentiate*** in the ***Evaluation*** module. *Result*: The ***Differentiate*** dialog opens.
2	• Select the desired source curve. • Select the ***First order*** calculation option. • Click ***OK***. *Result*: The differentiated curve will appear in the active chromatogram.
3	Select an appropriate Y-axis scale, right-click and select ***Vertical marker*** to measure the Y-axis values for the differentiated curve with the curve coordinates function. *Result*: The Y-axis value is interpreted as the UV curve slope at the selected retention point.
4	• Determine the highest slope value of the baseline (non-peak) part of the curve. • Add 10%. • Select ***Integrate:Calculate Baseline*** and use this value as the ***Slope limit***.

TIP: If the differentiated curve is very noisy, it can be filtered with a light ***Moving average*** filter in the ***Operations:Smooth*** function.

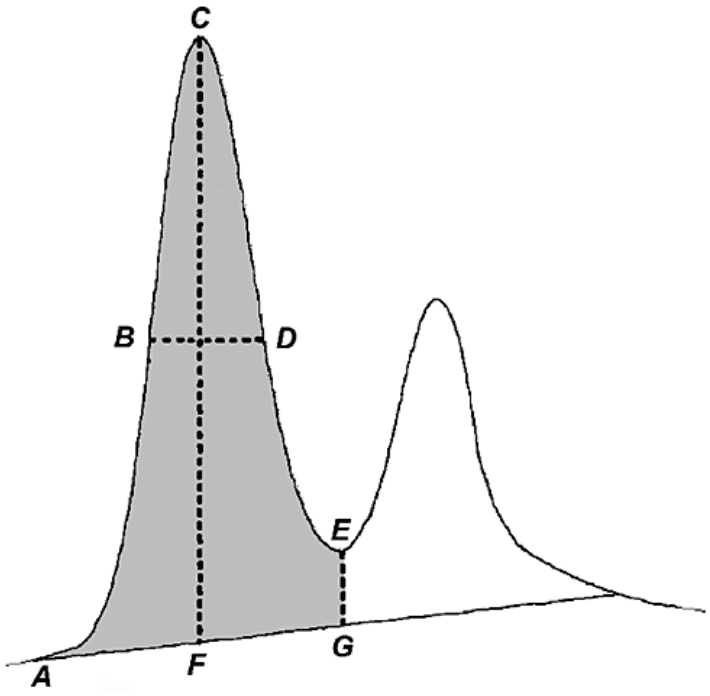
A.3 Peak table column components

Introduction

This section contains a list of peak parameters with explanations and calculation formulae when applicable.

Peak parameters - illustration

The diagram below illustrates the peak parameters. See the parameter list below for explanations.



Peak parameter descriptions

The list below contains descriptions of the peak parameters.

Parameter	Description
Area	Calculated as the area between the curve and baseline, between the peak start and peak end, time or volume base (gray area in the diagram above).
Asymmetry	Peak asymmetry (indicator of column packing). See definition below this table.
Baseline height	Baseline amplitude at peak start, peak maximum and peak end (A, F and G in the diagram above).
Retention factor	The retention factor will only be calculated when the chromatogram is in volume base. The total liquid volume, V_t, must be entered in the ***Peak Integrate*** dialog for this parameter to be calculated. See definition below this table.
Fraction tube id	Fraction number at peak start, peak maximum and peak end.
Height	Maximum amplitude above the baseline (C-F in the diagram above).
Kav	Gel phase distribution constant in gel filtration. Kav will only be calculated when a gel filtration column was used and when the chromatogram is in volume base. The void volume, V_0, must be entered in the ***Peak Integrate*** dialog for this parameter to be calculated. See definition below this table.
Plate height (HETP)	Height equivalent to theoretical plate and plates/meter. The column height must be entered in the ***Peak Integrate*** dialog for this parameter to be calculated. See definition below this table.
Peak endpoint heights	Amplitude above the baseline at left (A in the diagram above) and right peak limits (E-G in the diagram above).
Peak endpoint retention	Retention value at peak start and peak end, time or volume base (A and G in the diagram above).
Peak name	Name of the peak.
Percent of total area	Peak area as a percent of the total area under the curve above the baseline. Time or volume base. ***Note:*** *This value can differ in time and volume base if the flow rate is not constant throughout the method.*

Parameter	Description
Percent of total peak area	Peak area as a percent of the sum of all integrated peaks. ***Note:*** *This value can differ in time and volume base if the flow rate is not constant throughout the method.*
Resolution	Peak resolution. See definition below this table.
Retention	Retention at the peak maximum, time or volume base (C in the diagram above).
Sigma	Standard deviation for a Gaussian-shaped peak. See definition below this table.
Type of peak limits	Identifies the criteria for peak start and peak end as either the baseline intersection or dropline to the baseline or skim line.
Width	Difference in retention between the peak end and peak start, time or volume base (G-A in the diagram above).
Width at half height	Calculated by taking the maximum height of the peak above the baseline, then determining the peak width at half this value above the baseline. Time or volume base. (B-D in the diagram above, where BD bisects CF)

Note: *In the **Options** dialog (which is available in all UNICORN modules from the **Tools** menu) you can select if negative retentions should be displayed or not. The default selection is that negative retention is displayed.*

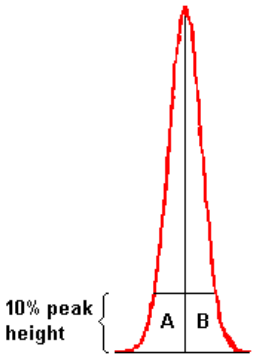
Asymmetry formula

The formula below is used to calculate the ***Asymmetry***.

Asymmetry = B / A

Where:

- A is a partial peak width, measured at a percentage of the peak height (asymmetry ratio), for the leading part of the peak.
- B is a partial peak width, measured at a percentage of the peak height (asymmetry ratio), for the tailing part of the peak.



Change the Asymmetry Ratio

The ***Asymmetry Ratio*** is selected in the ***Options*** dialog. The table below describes how to select a value:

Step	Action
1	• Choose the ***Tools:Options*** menu item. *Result*: The ***Options*** dialog opens.
2	• Type a ratio value in the ***Asymmetry Ratio at*** text box. • Click ***OK***. *Result*: The ratio value is changed and the dialog closes.

Note: *You must repeat the peak integrations after the change to update the values based on the new asymmetry ratio. The default ratio is 10%.*

Retention factor formula

The formula below is used to calculate the ***Retention factor***.

$$k^1 = \frac{V_R - V_t}{V_t}$$

Where:

- V_R = retention volume.
- V_t = total liquid volume.

Kav formula

The formula below is used to calculate ***Kav***.

$$k_{av} = \frac{V_R - V_0}{V_C - V_0}$$

Where:

- V_R = retention volume.
- V_0 = void volume.
- V_C = column volume.

HETP formula

The formula below is used to calculate the ***HETP*** value.

HETP = L/N

$N = 5.54 \times (V_R/w_h)^2$ assuming a Gaussian peak.

Where:

- N = no. of theoretical plates.
- L = bed height in cm.
- V_R = peak retention volume or time.
- w_h = peak width at half height expressed in the same units as V_R.

Peak resolution algorithms

The peak resolution is calculated with one of the following three algorithms:

1 $(V_{R2} - V_{R1}) / ((W_{b2} + W_{b1}) / 2)$

2 $(V_{R2} - V_{R1}) / ((Sigma_2 + Sigma_1) \times 2)$

3 $((V_{R2} - V_{R1}) / (2 \times (W_{h2} + W_{h1}))) / 2.354$

Where:

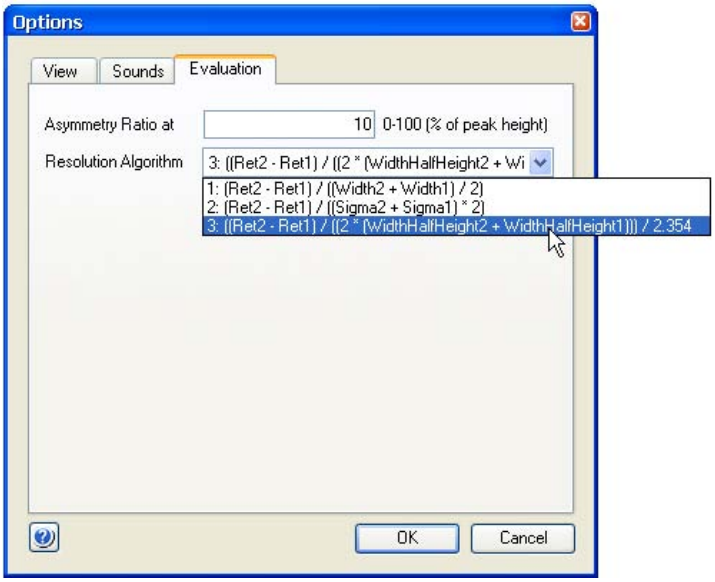
- V_{R1}, W_{b1}, $Sigma_1$ and W_{h1} are the retention, width, Sigma and width at half height of the previous peak.
- V_{R2}, W_{b2}, $Sigma_2$ and W_{h2} are the retention, width, Sigma and width at half height of the current peak.

Note: *The **Resolution Algorithm** variable in the **Options** dialog determines which of the three algorithms is used. The default setting is algorithm 3.*

Change the peak resolution algorithm

The table below describes how to change the peak resolution algorithm in the ***Options*** dialog.

Step	Action
1	• Choose the ***Tools:Options*** menu item in any of the UNICORN modules. *Result*: The ***Options*** dialog opens.
2	Click the ***Evaluation*** tab.
3	• Select the desired algorithm as described in *Peak resolution algorithm* above, in the ***Resolution Algorithm*** droplist.


Options
View
Sounds
Evaluation
Asymmetry Ratio at
10
0-100 (% of peak height)
Resolution Algorithm
3: ((Ret2 - Ret1) / ((2 * (WidthHalfHeight2 + Wi
1: (Ret2 - Ret1) / ((Width2 + Width1) / 2)
2: (Ret2 - Ret1) / ((Sigma2 + Sigma1) * 2)
3: ((Ret2 - Ret1) / ((2 * (WidthHalfHeight2 + WidthHalfHeight1))) / 2.354
OK
Cancel

- Click ***OK***.

Result: The dialog closes and the peak resolution algorithm is changed.

Note: *You must repeat the peak integrations after the change to update the values based on the new algorithm.*

Sigma formula

The formula below is used to calculate ***Sigma***.

$$\text{Sigma} = \sqrt{\frac{\sum_{i=1}^{n} \left(y_i \left(x_i - x_{ymax} \right)^2 \right)}{A_{peak}}}$$

Where:

- *n* is the number of data points.
- *x* is the volume or time value.
- x_{ymax} is the volume or time value at the maximum amplitude value.
- A_{peak} is the area of the peak.

Note: The peak width for a Gaussian peak is (4 x Sigma).

A.4 Evaluation procedure instructions

Introduction

This section contains information about the procedure instructions that are available for selection in the Instruction field of the ***Procedure Editor*** in the ***Evaluation*** module. Choose ***Procedures:Edit:New*** in the ***Evaluation*** module to view the ***Instruction*** list. The procedure instructions are used to build a complete, automated evaluation procedure, which may be included in a method and executed as part of a method run.

TIP:

- The mouse pointer is placed over a parameter field to show the allowed value range.
- The curve source is defined as the curve position number.

Curve operation

The table below contains a list of instructions for curve operations.

Instruction	Description
ADD	Adds two curves to produce a third curve, which is the sum of the two curves. The two source curves must have the same Y-axis unit and not be fraction, injection or run log curves, or else a run time error will occur.
AMP_MUL	Multiplies the amplitude of the source curve by the multiplication factor and stores the result in the target curve position.
AMP_SHIFT	Shifts the amplitude of the source curve by the shift factor and stores the result in the target curve position.
CLEAR	Clears the specified curve from the working memory of the computer.
COPY	Copies the source curve to the target curve position.
CUT	Cuts out the part of the source curve between the ***Left*** and ***Right*** limits and stores the result in the target curve position.
DERIVATE	Differentiates the source curve (first or second order) and stores the result in the target curve position. The Y-axis of the target curve position will be a normalized scale without unit.

Instruction	Description
DIV	Divides two curves to produce a third curve, which is the quotient of the two curves. The two source curves can have any Y-axis unit. The Y-axis of the target curve position will be a normalized scale without unit.
HISTOGRAM	Creates a histogram from any non-fraction curve (source curve 1) and a fraction curve (source curve 2_frac), and stores the result in the target curve position. If source curve 2 is not a fraction curve a run time error will occur. The Y-axis of the target curve position will be the same as that of the first source curve.
INTEGRATE	Performs a mathematical integration of the source curve and stores the result in a ***Result*** curve. This instruction is not the same as ***Peak integrate***, which performs a real peak integration.
RET_MUL	Multiplies the retention of the source curve by the **Multiplication** factor and stores the result in the target curve position.
RET_SHIFT	Shifts the retention of the source curve by the ***Shift*** factor and stores the result in the target curve position.
SMOOTH_AR	Smooths the source curve with an autoregressive filter and stores the result in the target curve position. The ***Filter*** parameter decides the strength of the filter.
SMOOTH_MA	Smooths the source curve with a moving average filter and stores the result in the ***Resulting Curve***. The ***Filter width*** parameter decides how many samples wide the filter is.
SMOOTH_MEDIAN	Smooths the source curve with a median filter and stores the result in target curve position. The ***Filter width*** parameter decides how many samples wide the filter is.
SMOOTH_SG	Smooths the curve with the Savitzky-Golay algorithm.
SUB	Subtracts two curves to produce a third curve, which is the difference of the two curves. The two source curves must have the same Y-axis unit and not be fraction or injection curves.

Instruction	Description
TDIV	Divides two curves to produce a third curve, which is the quotient of the two curves. The two source curves can have any Y-axis unit. The threshold values are used to avoid division of numbers close to zero. At those points where source curve 1 has an amplitude less than ***Threshold1***, or the source curve 2 has an amplitude less than **Threshold2**, the result of the division is defined to be 1.0.

Integration

The table below contains a list of instructions for integration.

Instruction	Description
CALCULATE_BASELINE	Calculates a baseline from the source curve. The baseline is stored in the target curve position. ***DEFAULT*** can be selected in the ***Baseline*** parameters, which will then calculate default baseline parameters for each new curve.
CALCULATE_BASELINE_MORPH	Calculates a baseline from the curve crvSrc using a morphological method. ***DEFAULT*** can be selected in the ***Baseline*** parameters, which will then calculate default baseline parameters for each new curve. The baseline is stored in curve crvDst.
CLEAR_PEAKTABLE	Clears the peak table in ***Peak table source*** from the computer memory.
COPY_PEAKTABLE	Copies a peak table from ***Peak table source*** to ***Resulting peak table***.
NEGATIVE_PEAKS	Controls the baseline behavior in subsequent baseline calculations. If ***ONOFF*** is **ON** then the baseline can be drawn above the curve and negative peaks can be detected by ***PEAK_INTEGRATE***. If ***ONOFF*** is ***OFF*** then the baseline is never drawn above the curve.
PEAK_INTEGRATE	Performs a peak integration on the source curve and stores the resulting peak table in ***Resulting peak table***. It is assumed that the baseline is subtracted.

Instruction	Description
PEAK_WINDOW	Specifies which part of the source curve that will be integrated. Peaks between retention ***Left*** limit and ***Right*** limit will be detected if the ***ONOFF*** parameter is set to ***ON***. If ***ONOFF*** is set to ***OFF***, the whole curve will be used for integration.
REJECT_PEAKS	Any combination of conditions is allowed. If all parameters are ***OFF*** then every detected peak is included in the peak table.
SET_COLUMN_HEIGHT	Sets the column height for the peak integration calculation of the ***HETP*** value. The ***Column height*** parameter is the height of the column in centimetres. If ***Column height*** is ***OFF*** then the ***HETP*** value is not calculated for the following integrations.
SET_COLUMN_V0	Sets void volume for ***Kav*** peak integration calculation.
SET_COLUMN_VT	Sets the total liquid volume for peak integration calculation of the capacity factor.
SET_SKIM_SIZE_RATIO	Sets the ***Skim size ratio*** to be used in the following peak integration(s).
WINDOW_PEAK_INTEGRATE	Integrates the curve within the peak window. All curve parts outside the peak window remain unchanged.

File operation

The table below contains a list of instructions for file operations.

Instruction	Description
CURVE_OPEN	Opens the curve specified in the ***Result*** file defined in ***File name*** and stores it in target curve position. If "*" is entered as ***File name*** the current result file will be used. The ***File name*** parameter may include a path from the users root folder.
IMPORT_CURVE	Imports a curve to the current chromatogram from another chromatogram (in the current file) and stores it in the target curve position.

Instruction	Description
IMPORT_PEAKTABLE	Imports a peak table to the current chromatogram from another chromatogram (in the current file) and stores it in the target curve position.
PEAKTABLE_OPEN	Opens the specified ***Peak table*** in the ***Result*** file defined in ***File name*** and stores it in the ***Resulting peak table***. If "*" is entered as ***File name*** the current ***Result*** file will be used. The ***File name*** parameter may include a path from the current users root folder.

Export

The table below contains a list of instructions for export operations.

Instruction	Description
EXPORT_CURVE_ASCII	Exports the curve entered in ***Curve source*** to the file defined in ***Export to file*** in ASCII. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If "?" is entered followed by text (e.g., "Enter a file name"), as file name, a full search path must be entered in answer to the question. In the part of the source curve limited by the ***Left*** limit and ***Right*** limit every <n> sample is exported.
EXPORT_DOC_400_ASCII	Exports the documentation in the current result file in ASCII format to the file defined in ***Export to file***. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If "?" is entered followed by text (e.g., "Enter a file name"), as file name, a full search path must be entered in answer to the question. If all parameters to this function are ***OFF*** then no documentation is exported. If at least one of them is ***ON*** then the documentation will be exported and the corresponding parts will be included in the exported file.

Instruction	Description
EXPORT_DOC_400_XLS	Exports the documentation in the current result file in MS Excel XLS format to the file defined in ***Export to file***. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If "?" is entered followed by text (e.g., "Enter a file name"), as file name, a full search path must be entered in answer to the question. If all parameters to this function are ***OFF*** then no documentation is exported. If at least one of them is ***ON*** then the documentation will be exported and the corresponding parts will be included in the exported file.
EXPORT_EVAL_LOG_ASCII	Exports an evaluation log in ASCII format to the file defined in ***Export to file***. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If "?" is entered followed by text (e.g., "Enter a file name"), as file name,, a full search path must be entered in answer to the question.
EXPORT_EVAL_LOG_XLS	Exports an evaluation log in Excel .xls format to the file defined in ***Export to file***. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If "?" is entered followed by text (e.g., "Enter a file name"), as file name, a full search path must be entered in answer to the question.
EXPORT_METHOD_ASCII	Exports a method to the file defined in **Export to file** in ASCII format. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If all parameters are ***OFF*** then no method is exported. If ***Main*** is ***ON*** then the main method is included and if ***Blocks*** is ***ON*** then all blocks are included in the exported file.

Instruction	Description
EXPORT_METHOD_XLS	Exports a method to the file defined in ***Export to file*** in Excel .xls format. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If "?" is entered followed by text (e.g., "Enter a file name"), as file name, a full search path must be entered in answer to the question. If all parameters are ***OFF*** then no method is exported. If ***Main*** is ***ON*** then the main method is included and if ***Blocks*** is ***ON*** then all blocks are included in the exported file.
EXPORT_MULTI_CURVES_ASCII	Exports multiple curves (previously defined with ***EXPORT_SEL_CURVES*** instructions) in ASCII format to the file defined in ***Export to file***. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If "?" is entered followed by text (e.g., "Enter a file name"), as file name, a full search path must be entered in answer to the question.
EXPORT_MULTI_CURVES_CSV	Exports multiple curves (previously defined with ***EXPORT_SEL_ CURVES*** instructions) in comma separated value .csv format to the file defined in ***Export to file***. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If "?" is entered followed by text (e.g., "Enter a file name"), as file name, a full search path must be entered in answer to the question.
***EXPORT_NORMALISE_*RETENTION	Normalizes retention when exporting multiple curves.
EXPORT_PEAKTABLE_ASCII	Exports the peak table in ***Peak table source*** to the file defined in ***Export to file*** in ASCII format. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If "?" is entered followed by text (e.g., "Enter a file name"), as file name, a full search path must be entered in answer to the question.

Instruction	Description
EXPORT_PEAKTABLE_XLS	Exports the peak table in ***Peak table source*** to the file defined in ***Export to file*** in Excel .xls format. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If "?" is entered followed by text (e.g., "Enter a file name"), as file name, a full search path must be entered in answer to the question.
EXPORT_PEAKTABLE_XML	Exports the peak table in ***Peak table source*** to the file defined in ***Export to file*** in XML format. If "*" is entered as file name in the ***Export to file*** field the current ***Result*** file will be used. If "?" is entered followed by text (e.g., "Enter a file name"), as file name, a full search path must be entered in answer to the question.
EXPORT_SEL_CURVES	Selects a curve for subsequent export (using the ***EXPORT_MULTI-CURVES_**** instruction). The curve is cut according to the right and left cut limit and the number of points to be exported may be set by the ***Export*** parameter (for example, every fifth point).

Chromatogram functions

The table below contains a list of instructions for chromatogram functions.

Instruction	Description
RESTORE_DESTINATION_CHROM	Resets the destination for the subsequent curve and peak table operations to the default chromatogram. Used together with the ***SET_DESTINATION_CHROM*** instruction.
SET_DESTINATION_CHROM	Opens the named chromatogram as destination for the subsequent curve and peak operations. Used together with the ***RESTORE_ DESTINATION_CHROM*** instruction.

Other instructions

The table below contains a list of instructions for other operations.

Instruction	Description
BASE	Sets the X-axis base that the following calculations will be made in. If the value of the X-axis base is ***DEFAULT***, then the default base is used (usually the base the method was run in). This instruction should be the first in the evaluation procedure, otherwise it will have no effect.
Comment	Inserts a comment below the marked instruction.
ENDLOOP	Marks the end of a ***LOOP*** statement.
LOOP	The instructions between this statement and the ***ENDLOOP*** statement are repeated n times. It is possible to have loops within loops as long as the number of ***LOOP*** statements matches the number of ***ENDLOOP*** statements.
QC_TEST	Performs a QC test according to the parameter settings.
REPORT	Prints a report with the specified named report layout and title. If ***Title*** is "*" then the title in the report layout is used. If ***Report Layout*** is "*" then a default layout is used.
RUN_PROGRAM	Starts a program as a separate process. The ***Program name*** string contains the program name and parameters to start it with.

Test instructions

The ***Instruction*** field also contains a group of test instructions. These instructions are only available for the UNICORN software development team.

Instruction	Description
AUTOSAMPLER_PEAK_INTERVALS	Sets the area intervals for the ***AUTOSAMPLER_PEAK_TEST***.
AUTOSAMPLER_PEAK_TEST	Locates the first peak in the peak table. Compares the area of the peak in the peak table with the specified maximum and minimum areas.

Instruction	Description
EXPORT_TEST_RESULT_TO_FILE	Finishes the current result and saves the output file as an ASCII file in a destination and with a file name specified in the variable DestFilename (.txt). A complete search path may be included in the file name.
GRADIENT_TEST_INTERVALS	Sets the level intervals for the ***NEW_GRADIENT_TEST***.
NEW_GRADIENT_TEST	The theoretical straight line between the 0% and 100% levels are calculated. The deviation between the curve and the ideal straight line is compared in both directions from the center position (50%) until the deviation exceeds the defined maximum deviation. The calculated deviation points are checked against the defined limits.
NEW_STEP_RESPONSE_TEST	The relative amplitude is calculated at the specified retentions (the 0% and 100% amplitudes are used for reference). The calculated relative amplitudes are checked against the specified error margins. The 0% level amplitude is verified to be within the specified interval from the absolute 0 level.
NEW_TEST_CURVE_AMPLITUDE_CHANGE	Verifies that the curve amplitude has changed more than or equal to the value of the Delta parameter between the defined to and from retention points. A print parameter may be set to On to generate printed results.

Instruction	Description
NEW_TEST_CURVE_AMPLITUDE_STABLE	Verifies that the curve amplitude is stable between the defined to and from retention points. The actual curve value is compared to a set amplitude parameter. If the difference exceeds a set Delta value, the test is failed. A print parameter may be set to On to generate printed results.
NEW_TEST_LOGBOOK_EVENT	Verifies if a specified text is present in the logbook curve between the defined to and from retention points. The test can be defined to be passed either if the text is present or not. A failed or passed text will be added to the output file. A print parameter may be set to On to generate printed results.
NEW_UV_RESPONSE_TEST	The amplitudes for the 0% and 100% levels are calculated and the difference between the values are calculated. The results of (1) Curve2_Difference / Curve1_Difference and (2) Curve2_Difference / Curve3_Difference are calculated. The calculated points are checked if they are outside the defined limits from the 50% level.
STEP_RESPONSE_INTERVALS	Sets the level intervals for the ***NEW_STEP_RESPONSE_TEST***.
TEST_INFO	Adds selected information items to the output file (e.g., system name), UNICORN version, etc. Also, a specified free text can be added. A print parameter may be set to On to generate printed results.
UV_RESPONSE_INTERVALS	Sets the level intervals for the ***NEW_UV_RESPONSE_TEST***.

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